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The Black Rootrot Disease of Apple

BY
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BLACKSBURG, MONTGOMERY COUNTY, VIRGINIA

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THE BLACK ROOTROT DISEASE OF APPLE

By F. D. Fromme [1]

The black rootrot disease of apple which is of rather common occurrence in Virginia was described and discussed in a previous paper by the writer, in collaboration with H. E. Thomas (1), in 1917. During the ten years that have passed further study of this disease has been in progress and considerable information has been obtained. The writer has been assisted at various times by members of the staff of the Department of Plant Pathology, and especially by S. A. Wingard and R. H. Hurt, whose contributions to the work are gratefully acknowledged. The scanty literature which existed prior to 1917 has been summarized in the previous paper, and that which has appeared subsequently will be discussed in connection with different phases of the problem.

THE CAUSATIVE AGENT

In our previous work the causative agent was determined to be a species of Xylaria which was thought at the time to be *X. hypoxylon.* The relation of other species of this genus to the disease was also considered. It is now definitely established that the first assignment of the species was in error. Mature fruiting stromata of the fungus, previously lacking, have since been found in abundance and their relation to the disease has been established. The correct designation of the species is still, however, somewhat uncertain.

The genus Xylaria, especially the North American species, has not been studied thoroughly in recent years, and there is some confusion as to the designation of species. The confusion is due in part to the application of names of European species to American material and in part to a lack of knowledge of the limitations of species. The species which commonly causes black rootrot of apple in Virginia is apparently identical with *Xylaria digitata* as this species was understood by Ellis, but it is quite uncertain that it is the *X. digitata* (L.) Grev. of Europe. The writer has examined European collections of this species in the Lloyd Hebarium, in that of the United States Department of Agriculture, and in the collections of Dr. C. L. Shear. The European species does not show the compressed, blunt tips which are characteristic of the species from apple and which are also featured in Ellis' description. The European species has ascospores 5-6 x 18-20μ while my measurements of the species from apple which are in exact agreement with those of Ellis for American material of *X digitata,* show ascospores 5-6 x 12-16μ. It seems rather certain, therefore, that the species in question is distinct from the species of Europe and is identical with that which Ellis described but erroneously referred to *X. digitata.* Since this name is properly applied to the species of Europe, and the American species is without a suitable name, it seems best to rename it and to present a more complete description than that given by Ellis. The following is a composite description of the species as

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it occurs on apple, and is based chiefly on a collection from Troutville, Virginia, October 11, 1921, which is illustrated in Figure 1.

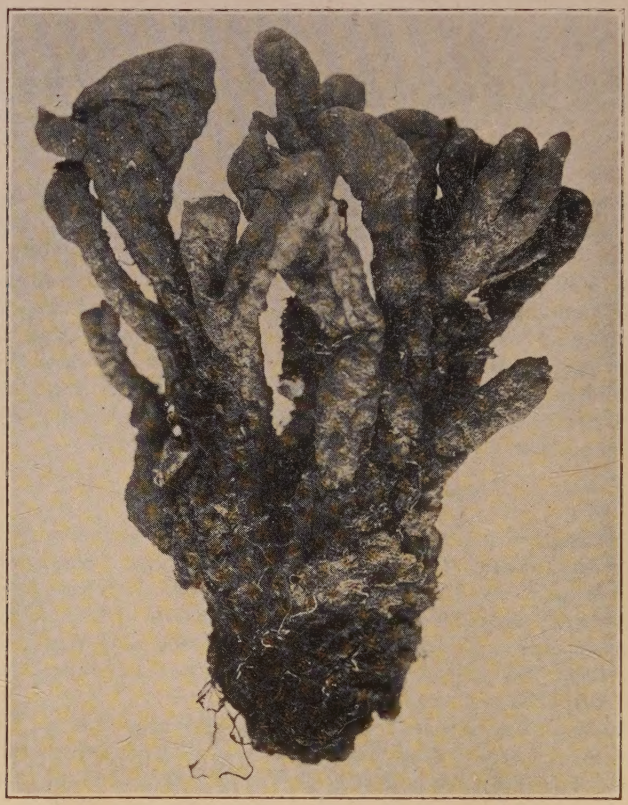

FIG. 1.—Stromata of *Xylaria mali* collected at base of diseased apple tree at Troutville, Virginia, October 11, 1921. Natural size.

Xylaria mali nom. nov.

X. digitata Ellis & Ev. N. Am. Pyrenomycetes, p. 669, 1892; not *X. digitata* (L.) Grev.

Stromata upright, united at the base, richly branched above into many furcate tips, entire mass fan-shaped, up to 10 cm. in height, about 8 cm. average, up to 8 cm. broad at the tip, 3 or 4 cm. in depth, dark-brown to blue-black in color, except for a mealy, white conidial hymenium which precedes the perithecia, glabrous except for compact purplish-black, iridiscent tomentum at base which may not be present in some specimens, surface smooth or considerably wrinkled; stromatic arms decidedly flattened, commonly 5 mm. or less in thickness, up to 2 cm. broad at the fork which forms two or more tips commonly about 1.5 cm. in length, tips tapering, usually conspicuously flattened or wedge-shaped; stipe up to 4 cm. in length, composed of several branches which are united at the base, sterile; conidia hyaline, ellipsoid with tapering ends, 3-4 x 8-10μ; perithecia

FIG. 2.—Stromata of *Xylaria mali* from dying apple tree at Greenwood, Virginia. The flattened tips are well shown. The specimen with the long stipe originated at an unusual depth. About natural size.

throughout except in the stipe and tips, deeply embedded and invisible at the surface except for the dots marking the ostioles, globose or sub-globose, about 700μ in average diameter, rather crowded, covered by a thick, hard layer of black stromata; context of stromatic arms dirty-white or yellowish, concolorous except for a prominent dark-gray band which subtends and surrounds the perithecia (the outer portion of the sporophore which consists of the crust, the perithecial layer and the pigmented zone is about 1 mm. in width); ascospores 8, overlapping, uniseriate, fusoid, slightly curved, dark-brown, 5-6 x 12-16μ, rarely

FIG. 3.—Stromatic arms of three species of Xylaria in longisection. A, *X. mali*. The perithecia are deep-seated and are embedded in a dark-grey band. B, *X. cornu-damae*. The perithecia are more superficial and the context is uniformly white. C, *X. longeana*. All are enlarged about 4X.

up to 18μ, mean 5 x 16μ. On roots and trunk at ground line of apple trees affected with black rootrot. Also occurring on oak and probably other deciduous woods.

The species is distinguishable by the compressed stromatic arms (Figures 1 and 2), the blunt tips, and the deeply embedded perithecia. The pigmented zone in which the perithecia are embedded does not occur, so far as I am aware, in other species, nor is it always noticeable in the species in question. It is well developed in some specimens, as shown in Figure 3, but in others which agree with the species in other features it may not be evident or only slightly so. It may be true that the degree of pigmentation varies with the maturity of the fungus or it may be a varietal or strain characteristic. There is ample evidence of the existence of distinct strains or varieties of the species. Their delimitations have not been clearly determined.

The writer has collected approximately one hundred specimens of *X. mali* in Virginia on apple trees affected with rootrot. The stromata are commonly found attached to the trunk and closely appressed to it with the stipe originating an inch or commonly two or three inches below the ground surface and the tips emerging above it. This is shown in Figure 6. Although rarely occurring in other positions, specimens have been found once or twice attached to a large root near the soil surface at some distance from the trunk, and they have sometimes been found on the broken ends of large roots left in the ground after the trunk had been removed. The clumps of stromata vary in size and number. Frequently there is only one group to a tree, but sometimes there are several, and rarely

an almost complete circle of them is formed around the trunk. They are especially apt to occur on the side which shows the most advanced injury. They commonly begin to form below the surface of the ground in the latter part of May, emerge in June, and produce conidia (Figure 7) during the latter part of June and early July. The production of perithecia and ascospores follows conidia production. The earliest collection showing mature ascospores made by the writer was one on September 12. It often happens that the stromata cease growth, shrivel and dry up without producing ascospores, although usually the primordia of the perithecia may be seen in specimens collected in late July and August. Specimens which were observed at frequent intervals in 1927 at Staunton were beginning to develop on May 23. They were then one-half inch in height, and were 3 inches beneath the surface. On June 5 they were emerging at the surface. They grew rapidly during the next two weeks and were producing a few conidia on June 18, but failed to produce ascospores. Stromata are usually found only on trees in late stages of rootrot or on those which have died recently. They rarely form during the early stages of infection.

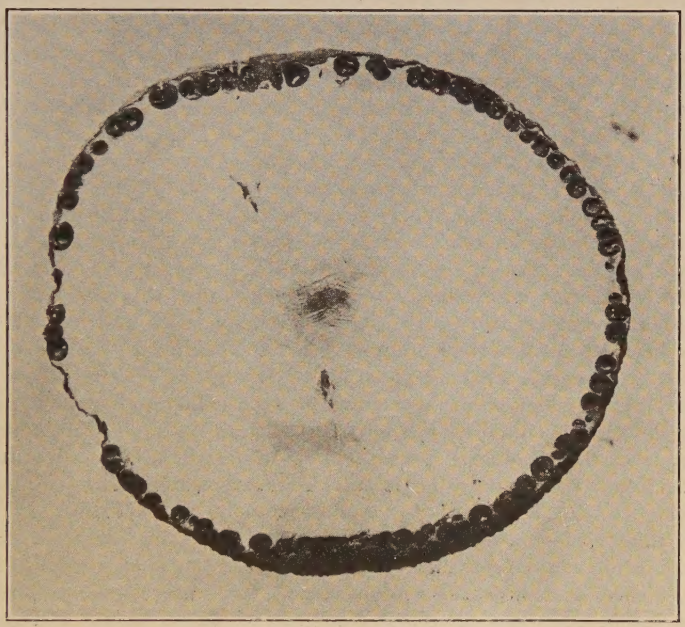

FIG. 4.—Stromatic arm of *X. polymorpha* in cross-section. About 4X.

Collections on apple have been made in Virginia in the counties of Albemarle, Augusta, Bedford, Botetourt, Buckingham, Clarke, Frederick, Giles, Halifax, Rappahannock, Rockbridge, and Shenandoah. A single collection was made by the writer on an oak stump in Augusta County, but no extended search has been made on trees other than apple. The writer has also seen immature specimens from apple collected at Clemson College, South Carolina; Milton, North Carolina; in Berkeley County, West Virginia, and at Farmington, Arkansas,

which appear to be *X. mali.* Its occurrence in southern Illinois is indicated from correspondence and by immature stromata sent to the writer by H. W. Anderson. It seems certain that the Xylaria rootrot of apple described from North Carolina by Wolf and Cromwell (6) and ascribed by them to Xylaria sp. is caused by *X. mali.* A culture of this fungus supplied by F. A. Wolf appeared identical with ours.

For some time there was a question in the writer's mind as to the possible identity of the species on apple with *Xylaria cornu-damae* (Schw.) Berk., a species which is not well known. Examination of the species in the Schweinitz Herbarium indicates the two to be distinct. A collection by Dearness (London, Ontario, September 23, 1892) which was issued as No. 2827 in Ellis and Everhart's North American Fungi appears to be representative of the Schweinitz material of this species. The stromata are commonly smaller, less richly branched and blacker in color than those of *X. mali,* and the prominent perithecia and larger ascospores are also distinguishing features. The appearance of a branch of the stromata in longisection is shown in Figure 3, B, and the ascospores in Figure 5, B. The writer has collected the species on *Robinia pseudo-acacia* at Barber, Virginia, and has grown it in culture. An interesting feature of this collection is that the ascospores were germinated and a culture established fourteen months after collection, the specimen having been stored in a dry condition meanwhile. The species is readily distinguished in culture from *X. mali.* It strongly resembles a culture previously isolated from apple roots from Harrisonburg which was referred to in our previous paper as Xylaria sp. Both this species and *X. mali* have commonly been referred to *X. digitata* by American collectors. For example, a collection from a pear tree which had died from an unknown cause which was referred to *X. digitata* by Weir (5) appears to be *X. cornu-damae,* as does also one by S. H. Burnham, Hudson Falls, N. Y., August 19, 1915. The writer has no evidence of the occurrence of this species on apple except the isolation referred to which cannot be considered a positive determination.

In addition to *X. mali* two other species of Xylaria, *X. polymorpha* (Pers.) Grev., and *X. longeana* Rehm, have been found in the fruiting stage on apple. The latter species, which occurs commonly on both fallen and standing dead wood, especially in eastern United States, has usually been referred to *X. hypoxylon.* It is distinct from the European species, which occurs in the United States only in the Pacific North-West, according to information available to the writer, and the name applied to it by Rehm, *X. longeana,* appears to be preferable. Our only collection of this species on apple was one at Blacksburg in which the sporophores were fruiting abundantly on the lower part of the trunk of a dead tree. There is little evidence that the species is parasitic. It occurs commonly on stumps of oak, black locust and other deciduous trees and on fallen wood. It is readily distinguished from the other species considered here, as shown by Figure 8, and is markedly distinct in culture since the mycelium is pink in color. Ascospores in the writer's collections are 4-5 x 9-12μ (Figure 5, C).

Xylaria polymorpha has been found by the writer on six trees of apple in two orchards, one in Alleghany County and one in Augusta. In the first orchard the sporophores were found on four trees affected with rootrot and in the Augusta County orchard two trees were affected. It was the sole species found in the first orchard but in the second it was of slight occurrence as compared with *X. mali.* The occurrence of this species on apple in New York is attested by Thomas (3) who finds it to be only slightly parasitic. It is frequently seen on stumps of such deciduous trees as black locust, butternut, black walnut, oak, hick-

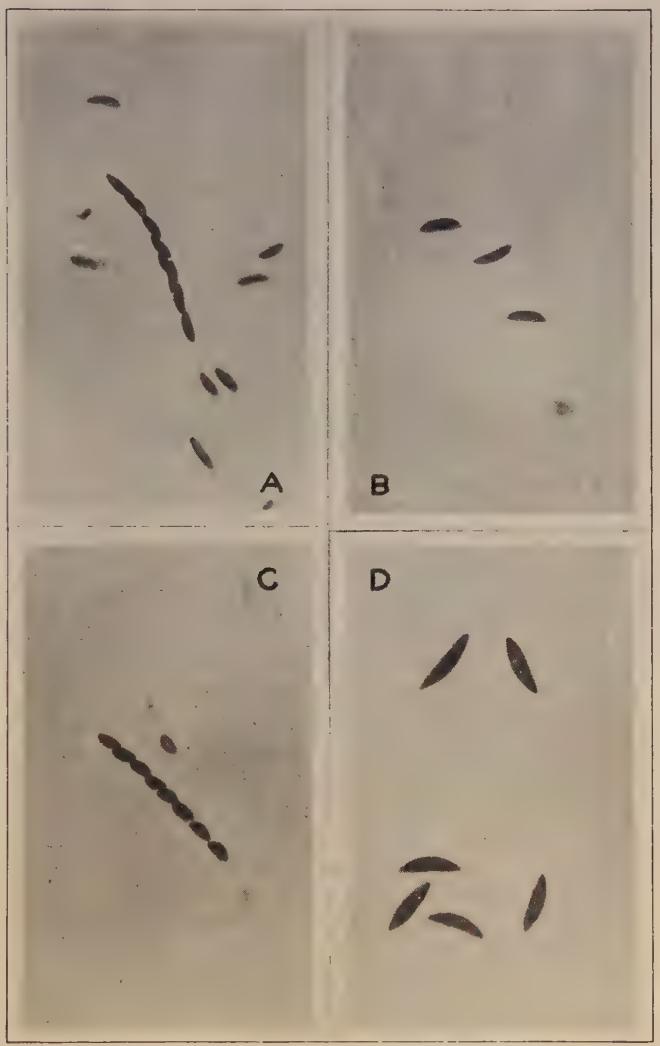

FIG. 5.—Ascospores of four species of Xylaria photographed at the same magnification. A, *X. mali*, 5-6 x 12-16μ. B, *X. cornu-damae*, 5-6 x 17-22μ. C, *X. longeana*, 4-5 x 9-12μ. D, *X. polymorpha*, 6-8 x 22-30μ.

ory, and maple and is sometimes popularly named "dead man's fingers." The few stromata that we have found on apple have been attached to an exposed root or one near the surface (Figure 9) at distances from the trunk varying from several inches to several feet. *Xylaria mali*, as has been noted, occurs almost without exception closely appressed and attached to the trunk. *X. polymorpha* is somewhat variable in appearance and distinct varieties have been recognized. It is

FIG. 6.—Stromata of *X. mali* in characteristic attachment to the trunks of apple trees that have died of black rootrot. The stipe originates beneath the soil surface and the tips emerge above it. The size of the group at the right is indicated by the half-dollar nearby.

not easily confused with any other species occurring on apple. Ascospores from apple material are 6-8 x 22-30μ (Figure 5, D). It is readily established and grown in culture from germinated ascospores or from mycelium from affected wood. The pathogenicity of the species will be discussed in another section of the report.

Behavior in Culture

Xylaria mali is readily grown in culture as are also the other three species which have been discussed. Cultures may be established from germinated ascospores or conidia, from fragments of the interior of young sporophores or from the invaded wood. Freedom from contamination is usually assured if wood fragments are plated from the advancing margin of the lesion on medium that is slightly acid in reaction. Satisfactory growth has been obtained on a variety of media, but Czapek agar, starch agar, corn meal agar, and steamed corn meal have been found most generally useful. The stromata which bear the conidial stage are commonly formed after a few weeks in culture, but no perithecia or ascospores have developed although a variety of media and conditions have been employed in attempts to produce them.

The four species are readily distinguished in culture, the more salient characteristics being the black pigmentation of older colonies of *X. mali*, the gray color

of *X. polymorpha*, the pink mycelial mat of *X. longeana*, and the white, fluffy colonies of *X. cornu-damae*.

The colony of *X. mali*, as illustrated in Figure 10, has a strongly zonated growth with alternating bands of appressed and aerial hyphae. Black pigment is produced in the older hyphae and eventually the surface of the entire mass is covered with a black incrustation. Stromata are produced readily and abundantly. They vary in size according to conditions of growth and abundance of food, but they are commonly about 10 cm. in height and about 4 mm. in width. They may become much longer than this with greater age and ample room and food for growth. The tips may be single or branched and are white and often mealy with conidia. The color darkens toward the base which is black and is usually enveloped in a mass of hair-like hyphae which are purple or brown in color.

The colony of *X. polymorpha* shows narrow, rather inconspicuous zones and is white on the margins and pale neutral gray in older parts. Stromata are readily produced and are gray in color with white tips and scanty brown hairs at the base. They are similar in height to those of *X. mali*, but are stouter as a rule.

The shrimp-pink color of the colony of *X. longeana* is a very distinct feature. The color diffuses into the medium. There is a considerable growth of aerial hyphae and little evidence of zones. Stromata are produced in abundance. They are quite small, usually 1.5 cm. long and 0.5 mm. in thickness.

The colony of *X. cornu-damae* is white or cream in color. No dark pigment is produced, as in *X. mali*, except a few slight patches at the center of the old colony. Stromata are slow in forming and few in number. The character of the mycelium has varied in our experience between different isolations. When colonies which had originated from single ascospores were transferred to separate plates they gave rise to two types of colonies. In one the hyphae were appressed, giving a flat smooth surface while the other had a fluffy cotton-like appearance due to the aerial growth of hyphae. Of nine ascospores plated, five produced colonies of one type and four the other.

There has been considerable variation in the appearance of isolations of *X. mali* from different sources. At one time the writer carried 24 isolations of this species from 14 different localities in culture for comparative study. Variation existed in the breadth and number of zones, in degree of pigmentation, in rapidity of stromata formation, in production of hairs and in other features, but it was not possible to group them in clearly defined strains. It is probable that strains of varieties exist within the species and there is some evidence of differences in pathogenicity.

OCCURRENCE OF BLACK ROOTROT

In general, black rootrot occurs practically coextensively with commercial apple production in Virginia. We have definite evidence of its presence in the counties of Albemarle, Amherst, Augusta, Bedford, Botetourt, Buckingham, Clarke, Franklin, Frederick, Giles, Halifax, Henry, Loudoun, Nottoway, Patrick, Prince Edward, Rappahannock, Roanoke, Rockbridge, Rockingham, and Shenandoah. No doubt it occurs in other counties contiguous to those named and within the general region which they embrace, but it is probably not an important disease in the state beyond this region. Our studies have been devoted to incidence and progress of infection in severely infested orchards rather than to geographic limits of occurrence.

FIG. 7.—Young stromata of *X. mali* that are mealy-white in color due to the abundant production of conidia. About natural size.

The disease is known to occur in states other than Virginia and according to present information the list of these would include Arkansas, Illinois, Indiana, Kentucky, North Carolina, South Carolina, Tennessee, and West Virginia. The writer has seen collections which may be assigned to *Xylaria mali* from most of these states and its occurrence in others seems assured from reports in the *Plant Disease Bulletin* and from correspondence. A map of the known occurrence in the United States is shown in Figure 11. It is reported from the eastern section of West Virginia, from seven counties in North Carolina, from Clemson College and Oconee County, South Carolina, from the counties of Adams, Hancock, and Henry in Indiana, from the states of Kentucky and Tennessee (widespread occurrence), from the counties of Jackson, Johnson, and Union in Illinois, and from Benton and from Washington Counties in Arkansas.

It is probable that the Xylaria rootrot of apple reported from New York and Pennsylvania is that with which *X. polymorpha* is associated, although *X. mali* may be expected to occur in the Cumberland Valley section of Pennsylvania and Maryland. It is *X. polymorpha*, according to Thomas (3), which occurs commonly on apple in New York while *X. mali* is unknown there. It would appear that the former species in its occurrence on apple has a more northern distribution than *X. mali*. It is known in Virginia on this host only from the counties of Augusta and Alleghany.

So far as the writer is aware, the black rootrot of apple does not occur except in the United States. Its presence in other countries where apples are grown seems doubtful since there has been no record of such occurrence so far as the writer can learn. The Xylarias, however, have generally been regarded

as saprophytes, and it is not inconceivable that the occurrence of parasitic species on apple or other hosts has been overlooked. Lyon (2) has described a destructive rootrot of Hibiscus which occurs in Hawaii and is caused by an undetermined species of Xylaria. Van der Bijl (4) has observed the fruiting of Xylaria sp. among dying plants of carnation in the Transvaal under conditions which suggested its parasitism. It is probable that inquiry would reveal additional cases.

SYMPTOMS OF ROOTROT

There are no symptoms of the aerial parts of the apple tree which are alone diagnostic for black rootrot. The roots also must be observed. Normally the growth of the tree is noticeably decreased for a period of a year, or commonly more, before death, the foliage is thin and off-color, single branches may die or exhibit pronounced symptoms of disease in advance of others, and the trunk may have a decided lean. Excessive blooming and fruiting usually accompanies the decline. A young tree in a late stage of rootrot is shown in Figure 12. None of these features is, however, exclusively diagnostic for black rootrot; they may equally well characterize decline due to other agencies. The presence of stromata of *X. mali*, in their characteristic attachment to the main root and trunk, is an unfailing evidence of black rootrot, as is also the characteristic black incrustation on the roots and the brittle, punky character of the wood throughout the invaded root. No other disease of apple roots affects them in like manner. Even though the black incrustation were lacking, the character of the affected wood would still be diagnostic. Because of its extreme brittleness even large trees in late stages of disease may usually be pushed over with little effort. The affected roots break off close to the main axis which is uprooted as a stub that has perhaps been supported by only one or two living roots (Figure 13). By the time that pronounced aerial symptoms of disease are shown the root system is usually almost

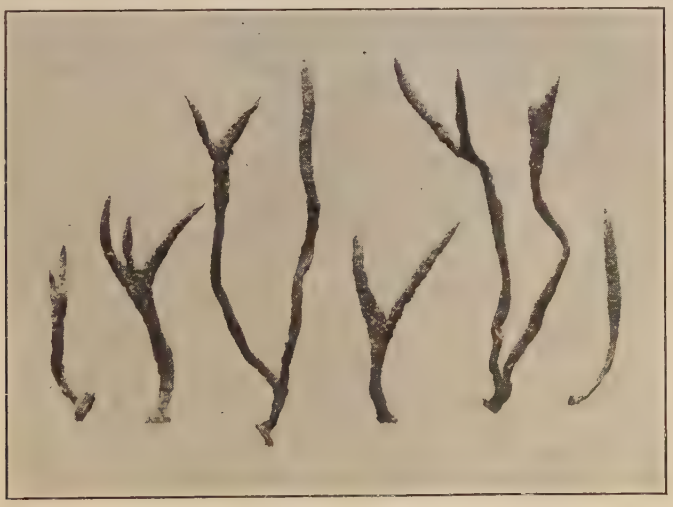

FIG. 8.—Stromata of *X. longeana* from the trunk of a dead apple tree. The species is non-pathogenic for apple. About natural size.

completely destroyed. When symptoms are exhibited by certain limbs and not by others the infection of roots corresponds to the location of the affected limbs. All tissues of affected roots are invaded and destroyed. The rot is a dry one: the root retains its general character until other organisms have destroyed it, but it quickly becomes brittle and punky enough to be broken easily or separated with the finger nail. The general color of affected wood is cinnamon-brown, the lighter portions being cinnamon-buff and the darker tawny-olive of Ridgeway's Color Standards. The marginal zone between affected and non-affected tissue is a much darker brown, almost black, and is hard and firm. Lesions on apple roots are shown in Figure 15, and the invaded area in Norway maple in Figure 16. The color lightens and the wood becomes less hard and firm in earlier invaded tissues. Dark lines or blocking layers are sometimes seen. The surface of the root is covered with more or less continuous patches of the black incrustation which is formed by the older mycelium (Figure 17). Here and there occasional newly-formed fans of white mycelium may be seen especially at the margins of the lesions but they are scanty and inconspicuous in the prevailing tone of black. The crusts are occasionally scanty and web-like but never form true rhizomorphs. There is no visible evidence of a mycelium in the soil apart from that which occurs on and within the roots.

The disease which we have called white rootrot occurs in occasional orchards in Virginia and has caused appreciable loss of apple trees in a few cases. We have definite evidence of its occurrence in the counties of Louisa, Campbell, Nelson, Montgomery, Patrick, Shenandoah, Wythe, and Smyth. It is not so common or so generally destructive as black rootrot from which it is readily distinguished by the dense mat of white mycelium which envelops the roots and crown. The character of the rot is also distinctive. The cortical tissues are invaded and are softened and disintegrated but the central cylinder remains hard and firm. Affected trees are not uprooted easily except when they have stood several years after death and other organisms have invaded the xylem elements. White rootrot often kills trees with great suddenness while they are apparently making vigorous growth. The crown may be invaded and completely girdled within a single season. The disease is especially prevalent in stump land and the period of maximum loss is usually between the ages of five and twelve years. The sporophores of a mushroom, *Clitocybe monadelpha* Morg., have been found at the base of affected apple trees and on nearby stumps of oak and chestnut. Although proof of pathogenicity has not been definitely established, the evidence strongly suggests that this fungus is the causative agent. The disease appears to be most prevalent in water logged soils that are acid in reaction. Improved drainage and liming have proved beneficial. The removal of soil from the region of the crown during the growing season has also aided recovery in some instances.

LOSSES AND DEVELOPMENT OF DISEASE

The incidence of apple tree death from black rootrot and other causes was determined for a limited number of orchards in Augusta and Frederick Counties in special surveys made in 1926.* The study included representative blocks of trees, ranging in number from 300 to 800, in commercial orchards which varied in age from 7 to 35 years.

In Augusta County the study included 13 orchards and a total of 7,189 trees, and in Frederick County 25 orchards and 13,029 trees. The occurrence, within the

*The survey in Augusta County was made by R. H. Hurt and that in Frederick county by F. J. Schneiderhan.

FIG. 9.—Stromata of X. polymorpha borne on a small root of a diseased apple tree at some distance from the trunk.

block surveyed, of trees that had died and been removed, of replanted trees, of dead and standing trees, and of sick trees was recorded and the most obvious cause of death or disease was assigned. Trees recorded as sick were those which were practically out of bearing and which would be expected to die in a year or so. The orchards are segregated in the tables in two groups, one in which rootrot did not occur or was of secondary importance, and the other in which it was the major cause of injury. The data for Augusta County are shown in Table 1.

In the six orchards where rootrot did not occur or was of minor importance the percentage of dead and sick trees ranged from 3.40 to 9.34 and averaged 5.76. For purposes of comparison this may be considered the average death of trees in non-rootrot orchards of 21 years, the average age. Mice were the chief causes of tree loss in orchards of this class. They were almost the sole cause in orchards Nos. 1, 2, and 5, and were the major cause with rootrot secondary in No. 3.

In the seven orchards where rootrot was the chief cause of death the percentage of dead and sick trees ranged from 6.88 to 21.94 and averaged 14.73. This figure, which may be taken as the average death in rootrot orchards of 18 years of age, exceeds the average death of non-rootrot orchards (5.76) by 8.97 per cent. and this figure may be considered roughly as the percentage of trees killed by rootrot. The average percentage of deaths and sickness in all 13 orchards was 10.53 at the average age of nineteen years.

It is true, of course, that one cannot determine with certainty the cause of death where a tree has been removed and destroyed, but the causes of current deaths are excellent guides, in the writer's experience, and the behavior of re-

TABLE 1

STAND OF APPLE TREES AND INCIDENCE OF TREE DEATH IN AUGUSTA COUNTY ORCHARDS

Orchard number	Original stand		Number of trees		Percentage Dead or Sick
	Age	Number	Dead	Sick	
Rootrot absent or of Minor Importance					
1	16	413	10	23	7.99
2	18	580	20	7	4.66
3	20	528	25	8	6.25
4	20	800	33	0	4.12
5	23	546	31	20	9.34
6	28	500	13	4	3.40
Average or total	21	3367	132	62	5.76
Rootrot of Major Importance					
7	7	320	18	4	6.88
8	11	300	34	0	11.33
9	14	636	30	15	7.08
10	18	500	44	10	10.80
11	18	674	84	20	15.43
12	22	720	151	7	21.94
13	35	672	136	10	21.73
Average or total	18	3822	497	66	14.73

plants is a further reliable guide. The cause of disease of the 128 trees which are classed as sick was determined as accurately as possible; 64 per cent. of the cases were attributed to rootrot and 36 per cent. to mice.

The findings of the survey in Frederick County are shown in Table 2. In the twelve orchards where rootrot did not occur or was of minor importance, the percentage of dead and sick trees ranged from 3.25 to 17.4 and averaged 10.83. The average age of trees was 21 years. A variety of causes for death were assigned, including mice, collar rot, blister canker, white rootrot, and the presence of rock ledges, as well as black rootrot which was present in slight amounts in some of the orchards.

In the thirteen orchards where black rootrot was the chief factor the percentage of dead and sick trees ranged from 7.50 to 44.4 and averaged 20.25. The average age of trees was 23 years. The difference between the average loss in the non-rootrot orchards and that in the rootrot orchards, which is 9.42 per cent., may be considered the percentage of trees killed by rootrot. Tree death and sickness in all 25 orchards averaged 15.82 per cent.

The picture from these two surveys is considered typical of the Shenandoah Valley fruit section. The losses from rootrot appreciably exceed those from any other single factor and equal or perhaps exceed the losses from all other factors. This is evident from the summary of data from both surveys which is shown in Table 3.

The tree loss in non-rootrot orchards was 9.04 per cent. while that in rootrot orchards was 18.28 per cent. The ages of the orchards compared are almost exactly equal. Each group averages 21 years. When one considers that these orchards are at the prime of bearing age, that they represent the average condition of the commercial orchards of the localities and are in no sense abandoned or neglected, the importance of the rootrot problem becomes apparent. In the

TABLE 2

STAND OF APPLE TREES AND INCIDENCE OF TREE DEATH IN FREDERICK COUNTY ORCHARDS

Orchard number	Original stand		Number of trees		Percentage Dead or sick
	Age	Number	Dead	Sick	
Rootrot absent or of Minor Importance					
1	12	300	14	6	6.67
2	13	400	9	4	3.25
3	15	600	43	3	7.67
4	17	600	80	20	16.67
5	18	400	30	4	8.50
6	20	600	74	16	15.00
7	23	600	45	4	8.17
8	25	600	33	7	6.67
9	26	400	32	8	10.00
10	27	500	80	7	17.40
11	30	600	53	9	10.33
12	30	529	57	26	15.69
Average or total	21	6129	550	114	10.83
Rootrot of Major Importance					
13	14	500	62	7	13.80
14	18	600	84	31	19.17
15	20	600	51	15	11.00
16	20	600	27	18	7.50
17	23	600	100	7	17.83
18	24	600	45	25	11.67
19	25	500	131	8	27.80
20	25	300	103	18	40.33
21	25	500	78	24	20.40
22	26	400	63	18	20.25
23	26	600	42	13	9.17
24	26	600	112	93	34.17
25	27	500	208	14	44.40
Average or total	23	6900	1106	291	20.25

TABLE 3

SUMMARY OF DATA ON STAND OF APPLE TREES AND INCIDENCE OF TREE DEATH IN 38 ORCHARDS IN VIRGINIA

Number and character of orchards	Number of trees		Percentage Dead or sick
	Original	Dead or sick	
18 Non-rootrot orchards	9,496	858	9.04
20 Rootrot orchards	10,722	1960	18.28
38 Orchards both types	20,218	2818	13.94

average orchard approximately 14 per cent. of the area is out of bearing at prime bearing age. In the average non-rootrot orchard, the loss is 9 per cent. and in the average rootrot orchard it is 18 per cent. In individual instances, the loss is much greater and it is in these that the importance of the disease is most apparent. In two of the 20 rootrot orchards the tree loss has exceeded 40 per cent.; it has exceeded 30 per cent. in one and 20 per cent. in five.

More detailed information on the incidence of tree death has been obtained by the writer in individual orchards. These studies have included an examination of all trees in several orchards and the causes of death and the progress

of disease have been determined with greater accuracy than in the general surveys. The records of three of these orchards will be presented. Orchards A and B are located in Augusta County and Orchard C in Frederick County.

Studies in Orchard A

The orchard in question is rather typical of a considerable number of orchards in Augusta County and other Valley counties. It is located near Staunton and has had somewhat better than average care. It was planted in 1903 and originally contained 2,110 trees with York Imperial the chief variety. About half the trees were set on newly-cleared land and about half on land that had been cropped for some years.

The writer's attention was first called to the orchard in 1916 through a complaint of tree loss from rootrot and a brief inspection was made in that year. A more detailed study and a map of the most severely infested part was made in 1917 and this was extended to include the entire orchard in 1918. Further studies and observations were made in the three succeeding years. The study was discontinued from 1921 until 1927, when a final complete check of the tree status was made.

While it is obvious that no accurate or complete record can be made of death of trees and the causes of death for the years prior to 1918, and for the years intervening from 1921 to 1927, it is still possible, however, to reconstruct a fairly accurate story of the loss of trees of the original stand from a study of the ages of replants. In doing this for the years where no other record exists it has been assumed that dead trees were removed promptly and were replanted the following year. According to the owner, this was the practise in earlier years.

The records indicate, as shown in Table 4, that death of trees began in 1909 at the age of seven years and that by 1927, at the age of 25 years, a total of 588 trees of the original stand had died, or 27.87 per cent. The loss is slightly more

TABLE 4
LOSS OF TREES OF THE ORIGINAL STAND IN ORCHARD A

Year	Age of trees	Death of trees		Cumulative percentage
		Number	Per cent.	
1909	7	3	0.14	0.14
1910	8	5	0.24	0.38
1911	9	10	0.47	0.85
1912	10	27	1.28	2.13
1913	11	24	1.14	3.27
1914	12	45	2.13	5.40
1915	13	46	2.18	7.58
1916	14	23	1.09	8.67
1917	15	18	0.85	9.52
1918	16	40	1.90	11.42
1919	17	22	1.04	12.46
1920	18	23	1.09	13.55
1921	19	57	2.70	16.25
1922	20	89	4.22	20.47
1923 to 1926*	21 to 24	154	7.30	27.77
1927	25	2	0.10	27.87
		588	27.87	27.87

*Of the 154 trees recorded for the years 1923 to 1926 seventeen were represented by replants in 1927, while the others were represented only by blank spaces. Thirteen of the replants were four years old and four three years old which indicates that thirteen original trees died in 1923 and four in 1924. The year of death for the other 137 trees could not be determined since no replants occurred in the spaces they had occupied.

FIG. 10.—A colony of *X. mali* on nutrient agar. The mycelium forms zones with fan-like margins. Pigmentation is beginning at the center. Eight-day development. About natural size.

than 1 per cent. of the trees for each year of growth and the death rate is rather uniform for ages from 10 to 25 years, although considerable fluctuation occurs, as would be expected.

Although it has been impossible to determine the causes of tree death, except for a small number of cases, because of the death and removal of trees prior to the beginning of our study and in intervals between visits, there can be little doubt but that the vast majority of deaths have been caused by rootrot. Eighty-two separate cases in all have been studied by the writer and of these 78 have been caused by rootrot and 4 by mice injury. Mice injury was rather prevalent in 1919. It was associated with rootrot in the death of 11 of the 22 trees that died in that year but was of primary importance in but one of these cases. In other years mice have been of slight importance. Aside from mice, there has been no other factor of any importance as a cause of tree death in this orchard except rootrot. It is a reasonable assumption that rootrot has been responsible for 95 per cent. of the deaths.

The course of disease in trees that have attained the age of 15 years or so is commonly marked by a steady decline exhibited over a period of several years. It is seldom that one observes affected trees of this age which do not show marked cessation of growth, death of large limbs, or other evidence of decline for at least a year or two prior to death. In Orchard A, 2 trees showed symptoms of disease for 7 years prior to death, 10 for 6 years, 18 for 5 years, 8 for 4 years, 9 for 3 years, 8 for 2 years, and 18 for 1 year. Stromata of *Xylaria mali* were found

on a considerable number of the affected trees. They were found on 23 of the original trees that died of rootrot during the period of observation and on five replants. The fungus would probably have fruited on other trees except for their removal late in disease or promptly at death. The stromata may appear a year or more in advance of death, in the year of death, or after death. In two cases they have appeared three years before death, but this is unusual. They do not form, as a rule, until a late stage of disease and often not until death or after. In one instance, they fruited on the same tree during three consecutive years.

Our experience with *Xylaria polymorpha* has been quite limited, but such evidence as we have indicates that duration of disease caused by this species is quite prolonged. Two trees in the orchard under discussion which have borne stromata of this species have shown symptoms of disease for three and four years. One of these recorded as three-fourths dead in 1919, when stromata were found on the roots, died during the winter of 1921-22. The other which appeared to be half dead in 1918, 1919, and 1920 was living in 1921, but died sometime during the period from 1922 to 1926. The fungus fruited on the roots of this tree in 1919, 1920, and 1921.

The history of replanting in Orchard A so far as it can be established is shown in Table 5. It is obviously impossible to accurately reconstruct the numbers of replants set in years prior to 1917 or in the years that intervened between 1921 and 1927. Without doubt many young trees have been planted, have died, and have been replanted a second or even a third time in the intervals that have elapsed. The record of replanting and replant deaths as given represents, therefore, merely the minimum numbers; the actual numbers no doubt exceed these appreciably.

TABLE 5
HISTORY OF REPLANTING AND REPLANT DEATHS IN ORCHARD A

Year set	No. set	1918	1919	1920	1921	1922	1923	1924-26	1927	Total dead	Per cent. dead	No. living 1927
1910	3							1		1	33.3	2
1911	5	1				1				2	40.0	3
1912	10	1							1	1	10.0	9
1913	27	1			1	2	1	6		11	40.7	16
1914	24	3				1	1	1	1	7	29.2	17
1915	45	1	4	2	1			9		17	37.8	28
1916	44	1	1	3	2	2		3		12	27.3	32
1917	22	9			1	1	1	3		15	68.2	7
1919	34			4	5	3	1	13	1	27	79.4	7
1921	29									0	0.0	29
1922	75								4	4	5.3	71
1923	123								3	3	2.4	120
1924	20									0	0.0	20
1925	6								1	1	16.7	5
	467	17	5	9	10	10	4	36	10	101	21.6	366

As shown in the table, a total of 467 replants have been set during the period from 1910 to 1925 and the death of 101 of these is recorded; 366 were still alive in 1927. The death of replants by 1927 is 21.6 per cent. of the total but a considerable number of the survivors have not reached the ages when death from rootrot is to be expected. It has been the experience in this and in other rootrot orchards that the greater number of replants die at about the age of five to seven years. As shown in Table 6, 43 deaths, 42.6 per cent. of the total deaths, occurred at these ages. The total ages of the 101 replants at the time of death

was 690 years and the average age at death was 6.8 years. Of the 467 replants only 214 would have completed seven years of growth in 1927 and the loss of these had been 93 trees, or 43.5 per cent.

TABLE 6
AGES OF REPLANTS AT TIME OF DEATH IN ORCHARD A

Age at death	No. of replants	Cumulative percentage
2	14	13.86
3	7	20.79
4	4	24.75
5	15	39.60
6	10	49.50
7	18	67.33
8	1	68.32
9	6	74.26
10	6	80.20
11	10	90.10
12	2	92.08
13	6	98.02
14	1	99.01
16	1	100.00

The death of replants, like that of the original trees, has been caused chiefly by rootrot. A few deaths have resulted from injury in cultivation, from the attacks of mice, and from lack of care. Such deaths usually occurred in the first or second years and removed trees which would probably have died at a slightly later age from rootrot.

Replants affected with black rootrot commonly die more quickly than older trees. The duration of disease increases with age. Young trees often die in the year that symptoms appear while older ones exhibit symptoms for a year or more before death. The young tree set in infested soil commonly becomes infected in a few years, and, because of its size, only a short period is required for the invasion and girdling of the main axis. Localized infections are often found on all or nearly all of the main roots (Figure 14). In its earlier growth on older trees, the fungus is often restricted for some time to one of the main roots and to branch roots in contact with it. Serious injury does not result until the main axis has been invaded. The infection of other roots at their origin on the main axis and the girdling of the latter follows eventually. The appearance of a diseased and healthy replant of the same age is shown in Figure 18.

Studies in Orchard B

This orchard is in Augusta County near Fishersville. The original trees, which were set in 1897, numbered 1,731, with the varieties York Imperial and Ben Davis predominating. The orchard was first visited and mapped by the writer in 1916 and a second survey was made in the fall of 1917. The trees were 20 years old in 1916. The tree deaths in earlier years have been reconstructed, as in other orchards, from the ages of replants, although it is obviously impossible to do this with accuracy for the earlier years in an orchard of this age. The tree loss as calculated for each year is shown in Table 7. Losses were especially severe during the period from 14 to 18 years of growth, the highest loss being at 17 years, when 59 trees, 3.41 per cent., died. The loss in 21 years was 367 trees, or 21.19 per cent. of the initial stand.

It has been possible to study replant death in this orchard only during the seasons of 1916 and 1917. While these studies give only a partial view of replant

losses, they indicate the high death rate which has occurred and which is typical of rootrot orchards. It is known that a number of the one-year replants that died in these years were the second replants at those locations. There is no method of determining the ages of the replants that preceded them. Replant

TABLE 7
LOSS OF TREES IN ORCHARD B DURING THE FIRST TWENTY YEARS GROWTH. THE ORIGINAL STAND CONTAINED 1,731 TREES

Year	Age of trees	Death of trees		Cumulative percentage
		Number	Percentage	
1904	8	3	0.17	0.17
1905	9	3	0.17	0.34
1906	10	2	0.12	0.46
1907	11	1	0.06	0.52
1908	12	2	0.12	0.64
1909	13	12	0.69	1.33
1910	14	58	3.35	4.68
1911	15	50	2.89	7.57
1912	16	39	2.25	9.82
1913	17	59	3.41	13.23
1914	18	52	3.00	16.23
1915	19	21	1.21	17.44
1916	20	44	2.54	19.98
1917	21	21	1.21	21.19
		367	21.19	

loss in the years 1916 and 1917 is shown in Table 8. The replant death in the two years is 14.24 per cent. of the number of replants standing at the time. The replant loss and that of the original trees was chiefly caused by rootrot.

TABLE 8
LOSS OF REPLANTS IN ORCHARD B IN 1916 AND 1917

Year of planting	No. of replants	Number died			Percentage of same age
		1916	1917	Total	
1905	3				
1906	3				
1907	2				
1908	1				
1909	2				
1910	12				
1911	58	4	3	7	12.07
1912	50	2	3	5	10.00
1913	39	3	3	6	15.38
1914	59	0	5	5	8.47
1915	52	5	8	13	25.00
1916	21	2	5	7	33.33
	302	16	27	43	14.24

Studies in Orchard C

This orchard is in Frederick County near Middletown. The original stand of 1,957 trees was chiefly York Imperial and Ben Davis. The trees were set in 1902 and were 15 years old when studied and mapped by the writer in 1916. The deaths in earlier years were reconstructed from the ages of replants. According

to the owner, the trees began to die of rootrot in a few years. The oldest trees which could clearly be recognized as replants in 1916 were then ten years old, indicating the death of the preceding trees of the original stand in 1906. According to the summary of tree deaths shown in Table 9, there were 9 deaths in 1906 with increased numbers in succeeding years. The maximum loss occurred in 1913 and 1914, when the trees were 12 and 13 years old. The number of deaths in each of these years, 94, was 4.8 per cent. of the original stand. The total tree loss, including the year 1916, was 469 trees, or 23.96 per cent. of the original stand. This is an annual loss of approximately 1.6 per cent. for each year of growth and represents a very severe condition when one considers that the trees had just attained good bearing age at the time of the survey. It would appear, from the conditions found in 1916, that a very large percentage of the tree loss has been caused by *Xylaria mali*. There was only a slight loss from white rootrot and other causes in that year. The loss of replants has also been severe. The replants numbered 416 in 1916, and 20, or 4.8 per cent., had died or were severely affected in that year. Replant death was due almost entirely to black rootrot.

TABLE 9

LOSS OF TREES IN ORCHARD C DURING THE FIRST FIFTEEN YEARS GROWTH. THE ORIGINAL STAND CONTAINED 1,957 TREES

Year	Age of trees	Death of trees		Cumulative percentage
		Number	Percentage	
1902	1			
1903	2			
1904	3			
1905	4			
1906	5	9	0.46	0.46
1907	6	9	0.46	0.92
1908	7	26	1.33	2.25
1909	8	18	0.92	3.17
1910	9	11	0.56	3.73
1911	10	20	1.02	4.75
1912	11	47	2.40	7.15
1913	12	94	4.80	11.95
1914	13	94	4.80	16.75
1915	14	87	4.45	21.20
1916	15	54	2.76	23.96
		469	23.96	

Susceptibility of Varieties

On theoretical grounds one should not expect to find appreciable differences in the susceptibility of varieties of apples to black rootrot, because of the similar type of root stock employed for all. It is generally assumed that the influence of the scion upon the stock is not such as would modify its resistance to disease, but the possibility of such influence must be admitted. There is also the further possibility that a portion of the root system that supports the tree, after the earlier years of growth, has been developed from scion wood. Certain varieties, notably the Northern Spy, develop roots from scion wood if the graft union is deep enough in the soil to permit their development. The degree to which this occurs in varieties is unknown but it is probably not uncommon. It is conceivable that the scion rooting of varieties may introduce differences in susceptibility which would not be evident if the root systems were developed in entirety from seedling roots.

The writer has made no extended inquiry into this phase of the problem, but certain data have accumulated which may have some significance.

Fruit growers have usually reported the York Imperial variety to be more commonly affected with rootrot than other varieties. This is to be expected since the York Imperial is the leading variety in Virginia in number of individuals. There is, however, some evidence from the deaths of varieties in Orchard A to support this belief. The varieties York Imperial, Ben Davis, and Missouri Pippin occur in appreciable numbers under comparable conditions in this orchard and the varieties Maiden Blush, Smokehouse, and Virginia Beauty are also represented but in insufficient numbers to justify comparisons. An analysis of the deaths of varieties in comparable areas is shown in Table 10.

TABLE 10
COMPARATIVE DEATH OF APPLE VARIETIES AT THE AGE OF 25 YEARS IN ORCHARD A

Variety	Location	Original number	Number dead	Per cent. dead
York Imperial	Rows 62-77	242	59	24.38
Ben Davis	Rows 58-62	103	13	12.62
York Imperial	Rows 46-55	212	67	31.60
Missouri Pippin	Rows 46-57	182	57	31.32
Virginia Beauty	Rows 9-18	28	5	17.86
Maiden Blush	Rows 11-18	46	9	19.56
York Imperial	Rows 9-18	189	62	32.80
Smokehouse	Rows 13-18	21	9	42.86

It will be noted that Ben Davis shows the lowest percentage of dead trees and a comparison with York Imperial is rather striking. The loss of Ben Davis in rows 58 to 62 is 12.62 per cent. The loss of York Imperial on one side of the Ben Davis block (rows 62-77) is 24.38 per cent., and that on the other side (rows 46-55) is 31.6 per cent. Missouri Pippin compared with the neighboring and interplanted block of York Imperial shows approximately the same percentage of loss. The varieties represented by few individuals show percentages of loss as follows: Virginia Beauty 17.86, Maiden Blush 19.56, and Smokehouse 42.86. In the same area York Imperial shows 32.8 per cent. loss.

INFECTION STUDIES

Proof of the transmission of infection through affected roots, infested soil and pure culture of *Xylaria mali* has been obtained in a variety of experimental tests. One of the early tests, which was begun by C. H. Crabill, demonstrated that infection may be established through contact with affected roots and infested soil.

Infection Through Root Contact and Infested Soil

A number of one-year apple trees of the York Imperial variety were planted in the month of April, 1915, in wooden tubs containing different soil mixtures and the tubs were partially buried out of doors. In one lot pieces of apple roots affected with black rootrot were placed in contact with the tree roots and the tubs were filled with soil from a cultivated field which was presumably free from the rootrot fungus. A second lot of trees was planted in tubs containing soil from a rootrot orchard. The soil was obtained from spots where trees had died of rootrot and was screened to remove apple root pieces. It was placed in the tubs in the summer of 1914 and was removed and reworked when the trees were planted.

The soil compost in which the third lot of trees was planted consisted of soil from a cultivated field and manure which had been inoculated with *Trichoderma koningi*, a fungus which is frequently isolated from rotted roots and which was considered at that time to be the probable causative agent. A lot of check trees was planted in part in the field soil and in soil with manure that had not been inoculated with Trichoderma.

The trees were harvested in the fall of 1917 after three growing seasons. As shown in Table 11 infection had occurred in one tree planted in contact with a diseased root and in two trees planted in infested orchard soil. There was no infection in the check lot or in the lot grown in the soil compost that had been contaminated with Trichoderma.

TABLE 11
BLACK ROOTROT INFECTION OF APPLE TREES GROWN IN TUBS AT BLACKSBURG, 1915 TO 1917

Sources of inoculum	Number of trees	Number infected	Percentage infected
Diseased apple roots	12	1	8.3
Infested orchard soil	15	2	13.3
Trichoderma koningi	8	0	0.0
Check	8	0	0.0

None of the infected trees had died at the time of harvest but death would undoubtedly have followed in a year or two. There were a number of localized lesions on the smaller roots as well as on certain main roots. Two roots, three-eighths inch and one-fourth inch in diameter were infected on the tree in contact with a diseased root piece. They had died back to the main axis which was one-third girdled at the time of harvest. *Xylaria mali* was isolated from the lesions. Infection was well established on one of the other trees on two roots, one-half and three-eighths inches in diameter, and on one root, three-eighths inch in diameter, on the other.

Since the tubs were only partially buried, the soil soon became very hard and dry. It is probable that more infection would have resulted if higher soil moisture had been maintained.

Infection Through Pure Culture Inoculation

Inoculation with *Xylaria mali* in pure culture with subsequent recovery of the inoculum has been successful in a large number of instances. The symptoms that have developed have been identical with those observed in naturally diseased trees and there can be no question as to the pathogenicity of the fungus or its relation to the causative agent in black rootrot. The first formal proof of this was obtained in a series of inoculations made on small apple trees at Blacksburg in 1926 and 1927. Although the percentages of infection that resulted were much lower than those obtained in subsequent experiments the results will be presented briefly.

The inoculum consisted of isolations of *Xylaria mali* from apple roots obtained from eight localities in Virginia. These are grouped as one lot in the data presented in Table 12. In addition to this species, two isolations of *X. polymorpha* and one of *X. longeana* were employed. The latter species was obtained from germinated ascospores from stromata which occurred on the trunk of a dead

apple tree. One culture of *X. polymorpha* was from ascospores from stromata growing on diseased apple roots and the other was an ascosporic isolation from stromata on a stump of black locust. A small portion of mycelium and the supporting medium was inserted into a cut made in the cortical tissues of the root. The cut was not wrapped to prevent drying, as in the later work, and it is probable that the low percentages of infection were due in part to the rapid drying of the inoculum.

One-year grafts of York Imperial were used in the plantings made May 1 and December 4, 1916, and ungrafted nursery seedlings were used in that of April 20, 1917. The soil in which the trees were set was rather shallow and of low fertility, a condition which is not favorable for rootrot development.

In all, 310 trees were inoculated with *X. mali*, 33 with *X. polymorpha*, 10 with *X. longeana*, and 118 served as checks. The latter were wounded like the inoculated trees.

All trees were harvested in April, 1919. The numbers and percentages of infected trees are recorded in Table 12. The percentage of trees infected with *X. mali* ranged in the three lots from 3.3 to 5.2 and averaged 4.2. One tree had died of rootrot prior to harvest and the infection in the others ranged in degree from slight to severe. One tree of the 15 inoculated in 1916 with *X. polymorpha* from apple showed a slight infection while the lot inoculated with the same culture in 1917 showed no infection, as did also that in which the isolation from black locust was used. There was no infection in the lot inoculated with *X. longeana* or in the check lot.

TABLE 12

INFECTION FROM APPLE TREES RESULTING FROM PURE CULTURE INOCULATION WITH SPECIES OF XYLARIA AT BLACKSBURG, 1916 TO 1919

Inoculum	Date	Number of trees		Percentage infected
		Inoculated	Infected	
X. mali	May, 1916	55	2	3.6
"	December, 1916	135	7	5.2
"	May, 1917	120	4	3.3
X. polymorpha (apple)	December, 1916	15	1	6.7
" (locust)	December, 1916	8	0	0.0
" (apple)	May, 1917	10	0	0.0
X. longeana	May, 1917	10	0	0.0
Check	1916 and 1917	118	0	0.0

A further test of the susceptibility of apple and of a limited number of other deciduous trees which were considered possible hosts for *X. mali* was begun at Blacksburg in 1922.

The inoculum consisted of three isolations of *X. mali* from apple roots and one of *X. longeana* from stroma from an oak stump. A liberal piece of the mycelial mat from the margin of a vigorous culture was inserted behind a flap cut through the cortical tissues of the root and extending slightly into the wood cylinder. The wound was wrapped with a thin strip of moist absorbent cotton and the tree was planted at once. One inoculation was made to a tree; on small trees low on the main axis and on larger trees on the largest branch root an inch or two from the main axis. Checks were wounded in like manner. The planting period extended from April 20 to April 25, 1922. The trees were kept under clean cultivation during the first two years of the test, but received only infrequent cultivation from then until the final harvest in February, 1927.

The varieties of trees employed and the numbers inoculated and used as checks are shown in Table 13. All were one-year seedlings except the trees of butternut, chestnut, Norway maple, and silver maple which were older than one year. The apple material consisted of two lots of seedlings, one grown from seed imported from France and the other from American seed.

TABLE 13
SPECIES AND NUMBERS OF TREES INOCULATED AND USED AS CHECKS IN TESTS AT BLACKSBURG, 1922

Species of tree	Number inoculated with		Number of checks
	X. mali	X. longeana	
Apple, American seed	232	61	73
Apple, French seed	130	20	15
Ash, *Fraxinus americana*	60	20	20
Butternut, *Juglans cinerea*	6	2	2
Walnut, *Juglans nigra*	59	21	20
Cherry, *Prunus mahaleb*	70	24	10
Chestnut, *Castanea dentata*	6	2	2
Elm, *Ulmus americana*	54	24	20
Honey Locust, *Gleditsia triacanthos*	60	19	20
Locust, *Robinia pseudoacacia*	60	14	20
Norway Maple, *Acer platanoides*	6	2	2
Silver Maple, *Acer saccharinum*	5	2	1
Box Elder, *Acer negundo*	60	18	18
Pear, *Pyrus communis*	72	15	10
Pear, *Pyrus ussuriensis*	73	15	11

Dead trees were harvested at intervals throughout the duration of the test which was completed in February, 1927. Infection with *X. mali* which varied appreciably in intensity was obtained on apple, cherry, elm, honey locust, pear, and Norway maple. There was no infection of ash, black locust, box elder, butternut, chestnut, silver maple, or walnut. *Xylaria longeana* produced infection only on cherry. The results of the inoculations are summarized in Table 14. Only those species which were infected are included in the table and in these the inoculations with *X. longeana* are omitted except where infection occurred. The details of infection are discussed separately for each species.

There was a high death rate in the apple seedlings during the first year due in part to infection and in part to conditions of growth. The season was dry and the trees were small and easily killed by exposure. There was little apparent difference between the inoculatd lots from American and French seed. Both showed high percentages of infection and death.

Of the 146 American seedlings that died, 84 were infected and in the majority of them infection was severe and had clearly been responsible for death. The tops of a number of the trees withered and died after planting but the roots of these were generally living and sound at the end of the season except for infection with *X. mali.* Only one infected tree of this lot survived beyond the first season. The survival of trees of the check lot and of that inoculated with *X. lengeana* is useful here as an index of the importance of *X. mali* in the death of the trees. The loss of trees of the check lot was 27.4 per cent., that of the lot inoculated with *X. longeana* (of which none was infected) 29.5 per cent., and 26.7 per cent. of those inoculated with *X. mali* died but showed no infection.

Of the 130 French apple seedlings inoculated with *X. mali,* 67 died during the first season and 55 of these showed well established infection. Two others were dead from black rootrot when dug in April, 1923, and two others showed severe infec-

TABLE 14

INFECTION OF SEEDLINGS OF APPLE AND OTHER SPECIES RESULTING FROM INOCULATION WITH SPECIES OF XYLARIA AT BLACKSBURG, 1922-1927

Species of tree	Inoculum	Number of trees			Percentage	
		Set	Died	Inf.	Died	Inf.
Apple, American seed	*X. mali*	232	146	84	62.9	36.2
"	check	73	20	0	27.4	0.0
Apple, French seed	*X. mali*	130	69	59	53.1	45.4
"	check	15	2	0	13.3	0.0
Cherry, Mahaleb	*X. mali*	70	48	34	68.6	48.6
"	*X. longeana*	24	14	12	58.3	50.0
"	check	10	2	0	20.0	0.0
Pear, P. *communis*	*X. mali*	72	5	5	6.9	6.9
"	check	10	0	0	0.0	0.0
Pear, P. *ussuriensis*	*X. mali*	73	4	1	5.5	1.4
"	check	11	0	0	0.0	0.0
Honey locust	*X. mali*	60	6	3	10.0	5.0
"	check	20	0	0	0.0	0.0
Norway maple	*X. mali*	5	0	5	0.0	100.0
"	check	2	0	0	0.0	0.0
Elm	*X. mali*	54	14	12	25.9	22.2
"	check	20	0	0	0.0	0.0

tion when dug at the end of the test. Infection in this lot was 45.4 per cent.

Both species of Xylaria produced a rather vigorous infection of Mahaleb cherry associated with a high rate of death. Since all deaths occured during the first season it seems likely that poor conditions for growth were responsible in some measure for the rapid death of the inoculated trees. Most of the trees that died showed no growth of the tops but the roots were usually alive at harvest except those that were infected. Of the 70 trees inoculated with *X. mali*, 48 died and 34 were infected. Fourteen of these showed vigorous infection with conspicuous development of the black incrustation and extensive rotting of the wood. The inoculum was apparently the chief factor in the death of these trees. The other 20 trees showed positive infection but it had not progressed far from the vicinity of the wound. Twelve trees (50 per cent.) were infected with *X. longeana*, seven showing vigorous development of infection with extensive invasion of the wood while five showed less extensive infection. There was death of 20 per cent. of the check trees while that of the *X. longeana* lot was 58.3 per cent., and that of the *X. mali* lot 68.6 per cent.

A small percentage of the pear seedlings showed infection. Only one of the lot of *Pyrus ussuriensis* was infected and this was of a minor character. The tree together with two others of the same lot died during the first season. Infection of *P. communis* was of a more definite character. The five infected trees died during the first season. The wood near the wounds was punky and brittle and there was extensive development of the black crust. None but the five infected trees died during the course of the test.

Of the 60 trees of honey locust inoculated with *X. mali*, four died in the first season and two were infected, one slightly, and the other severely. Two others died in the second year, one showing severe infection which was clearly responsible for death. The other was dead in the top, only the roots being alive and free from infection. All of the check trees were in vigorous condition at the end of the test.

Norway maple alone of the three species of Acer showed infection with *X. mali*. All five inoculated trees became infected. Four showed very vigorous infection and except for their large size and extensive root development all would

probably have died before the completion of the test. The effect of the disease was exhibited in the tops which were less vigorous than those of the checks. Three of them were harvested in February, 1925, two showing vigorous infection with complete invasion of the inoculated branch root and about half of the main axis (Figure 16). The inoculated root of the third was invaded about two inches on either side of the wound. The two remaining trees when harvested in February, 1927, were dwarfed and almost dead. Only a few small roots high on the main axis were alive and supporting growth. All other roots and the lower part of the main axis were infected and dead. The invaded wood of Norway maple does not become soft so rapidly as does that of apple but eventually the same condition is reached. Near the margin of the lesion the wood is light brown with a greenish-yellow band on the border of the non-infected wood. The color is a somewhat darker brown in older parts of the lesion and occasional irregular black lines occur. The pathogen was isolated without difficulty from several of the trees and one isolation was used later to establish infection in apple. The two trees inoculated with *X. longeana* and the two checks were healthy and vigorous at harvest.

Twelve trees of elm developed infection from *X. mali*, a percentage of 22.2. All of these died during the first season and these, together with two additional trees which were not infected, constituted the total deaths in this lot. There was a rather extensive development of the black crust on the roots of the infected trees but the penetration of the wood appeared to be slight and there was some doubt as to the role played by the pathogen in the death of the trees. Six of the 10 trees inoculated with *X. longeana* died during the first season but none showed infection. All other trees of this lot and all checks were healthy at harvest.

Studies With Scion Roots of Apple Varieties

It had seemed apparent almost from the beginning of our work with black rootrot that the most promising approach to the problem of control would lie in the development of a resistant root-stock. Several possibilities were suggested, including the use of species of Malus other than the cultivated apple, and the propagation of varieties or individuals by asexual methods. Almost all of the apple trees produced by American nurserymen are grafted on seedling roots of the cultivated apple, *Malus malus*. Seedlings in large quantities were formerly imported for this purpose but this supply has been replaced in recent years with American grown seedlings. Some of these are grown from imported seed and others from home-grown seed with the tendency towards increased production of the latter. There is probably little, if any, difference of moment between the seed of foreign and home origin. Both are obtained from pomace of mixed varieties of the cultivated apple, and although the mixture of varieties differs the seedlings have similar ranges of variability. If the horticultural varieties of apple differ in susceptibility to rootrot, one would expect to find these differences in all degrees in mixed seedling lots and to some degree in seedlings of individual varieties, since the latter do not breed true for other qualities. Because of these considerations it had seemed unwise to seek for resistance in commercial lots of seedlings or in seedlings of varieties, although rather extensive tests of these were made later in the course of the work. The variety or individual capable of asexual propagation seemingly offers greater possibilities.

An opportunity to test the relative susceptibility of certain varieties was provided through the courtesy of Dr. J. K. Shaw, who sent us some trees that he had propagated on scion roots. The scions had been grafted in the usual way on a seedling nurse root which had been removed on the development of roots from the

scion. The varieties represented were Ben Davis, Maiden Blush, Newtown Pippin, Northern Spy, Red Astrachan, and Stayman. The trees were three or four years old when received. They were inoculated and planted at Blacksburg on May 11, 1917. The inoculum consisted of a culture of *X. mali* obtained from Middletown, Virginia, in 1915. It was inserted behind a flap cut in the largest branch root at a point about two inches from the crown. The check trees were also wounded.

Because of the rather late planting date and the dry weather which followed a number of the trees died in the tops during the first season. When harvested on October 15, the roots of these were alive and sound except where infection had resulted. All the remaining trees that survived the first season were harvested April 1, 1919. A summary of the results obtained is shown in Table 15 and the degree of infection secured with each variety is discussed separately in the following paragraphs.

FIG. 11.—Occurrence of black rootrot of apple caused by *Xylaria mali*. Each dot indicates a county or locality of known occurrence.

Newtown Pippin.—Four trees were inoculated and one served as a check. The tops of all five died during the first season but such roots as were not invaded by the pathogen were sound when harvested in October. All inoculated trees showed a vigorous development of disease. Infection had progressed to the main axis in all and had become well established. One was completely girdled. The roots of the check were sound and alive. The infection of this variety was more uniformly vigorous than that of any other tested. Although drought, occasioned in part by a rather scanty root system, was primarily responsible for the death of the tops it seems probable that the infection was a contributing factor. It is likely that none of the infected trees could have survived more than two seasons.

Maiden Blush.—Six trees were inoculated and two served as checks. Four inoculated trees died in the tops following transplanting and three showed vigorous infection of the roots and one a slight infection. The main axis had been invaded in three and in one of these it was completely girdled. None of these would probably have survived a second season. The two remaining inoculated trees survived until the end of the test. One showed vigorous infection with the main axis half girdled while the other showed no infection. Both checks died

the first season. The roots of one were alive, while those of the other were dead with a type of rot not characteristic of Xylaria.

Red Astrachan.—Seven trees were inoculated and two served as checks. One of the inoculated trees died during the first season. It had produced leaves, but these had withered and died. The roots were living except for affected parts and a vigorous infection had resulted. The fungus was well established in the main axis which was half girdled. The remaining six inoculated trees were harvested at the end of the test. One showed no infection, three showed only slight infection, and two showed vigorous infection. The infection in those recorded as slight had extended only a little beyond the wound, the most extensive lesion being only 2 cm. in length and involving half of the root in depth. At this rate of progress the infection would seemingly have caused no appreciable injury during the normal life of the trees. The condition presented by the two trees with vigorous infection was in sharp contrast. One of these could not have survived a third season and the other would probably have died either in the third or fourth season. The main axis of one was girdled in a band varying from 6 to 12 cm. in breadth and all of the branch roots had developed infection through their origin in the main axis (Figure 19). The main axis of the other tree was half girdled and infection had resulted in three-fourths of the branch roots. Both checks were living and free from infection at the end of the test.

Stayman.—Seven trees were inoculated and two served as checks. Five of the inoculated trees and both checks died in the tops during the first season. One of the inoculated trees showed vigorous infection, the crown being half girdled. It had made some growth during the early season but the leaves had withered during midsummer and it is probable that the infection contributed to the death of the tree. The infection of the other four trees was slight but the fungus was active and was extending the affected areas slowly. Both roots and tops of the check trees were dead when harvested but there was no Xylaria infection of the roots. The two remaining inoculated trees were harvested in April, 1919. One showed slight infection which extended 4 cm. on either side of the wound and penetrated half-way through the root. The inoculated root of the other was dead and the fungus was established in the main axis at the point of origin of the root.

Ben Davis.—Seven trees were inoculated and two served as checks. One inoculated tree did not survive transplanting and showed slight infection at the wound. All others were free from infection and in vigorous condition at the end of the test.

Northern Spy.—There were eight inoculated trees and two checks. All grew well the first season and they were not disturbed until the end of test in the

TABLE 15
SUMMARY OF INFECTION OF SCION-ROOTED APPLE TREES WITH XYLARIA MALI AT BLACKSBURG, 1915-1917

Variety	Number of trees			Percentage	
	Inoc.	Inf.	Vig. Inf.	Inf.	Vig. Inf.
Newtown Pippin	4	4	4	100	100
Maiden Blush	6	5	4	83	67
Red Astrachan	7	6	3	86	43
Stayman	7	7	2	100	29
Ben Davis	7	1	0	14	0
Northern Spy	8	0	0	0	0

spring of 1919 when they were harvested, examined and replanted for further work. No infection had occurred. The wounds had healed normally and the roots and tops had made vigorous growth.

FIG. 12.—A young apple tree in a late stage of black rootrot. The thin foliage and practical death of limbs on the right side indicate that the majority of roots on this side have been destroyed.

The Northern Spy was the outstanding variety of those tested from the standpoint of freedom from infection, while Ben Davis developed only slight infection of one tree. Newtown Pippin was the most susceptible, with Maiden Blush, Red Astrachan, and Stayman showing decreasing susceptibility in the order named. Northern Spy was the only variety in which all trees survived the shock of transplanting and grew vigorously throughout the period of the test. Since it had no preferential treatment, it would appear to be drought resistant as well as resistant to Xylaria. Its resistance to drought is no doubt determined by the vigor of its scion-root development. It has been known for some years as a variety that produces scion-roots readily. The question may be

raised as to whether the death of tops in some of the varieties may not have contributed to the infection of the roots, and possibly this was a factor. If Northern Spy and Newtown Pippin alone are compared one might conclude that death of the shoot was responsible for vigorous infection of the root, but comparison between Ben Davis and Red Astrachan does not support such a view. Although both varieties lost only 11 per cent. through death of the shoot, Red Astrachan developed 86 per cent. of infection and Ben Davis only 14 per cent. In these varieties at least there seems to be inherent differences in susceptibility that are not modified materially by the nutrition of the root.

A comparison of the susceptibility of scion roots of Northern Spy with seedling roots of nursery stock was made in 1919. The Spy-root trees, which were obtained from a nursery, were one-year buds of Jonathan on scion-roots of Northern Spy. The other trees were one-year grafts of York Imperial on nursery seedlings.

The inoculum consisted of an isolation of *Xylaria mali* from apple roots obtained at Greenwood, Virginia. Two flaps were cut into the roots of each tree, one in the main axis and the other in the largest branch root. Check trees were wounded in like manner. The inoculum was inserted in the wound which was then wrapped with moist absorbent cotton. The trees were inoculated and planted April 9, 1919, at Crozet, Virginia. There were 23 inoculated trees and 8 checks on Spy roots and 37 inoculated trees and 14 checks on seedling roots.

The trees were examined on July 18, 1919, approximately three months after planting, and all those with shoots that had failed to grow or which had died in the interim were harvested. All of the Spy-root trees were alive, but 15 of the seedling-root trees (13 inoculated and 2 checks) were dead. The shoots of these trees had either failed to start or had made only a slight growth before death which was due primarily to drought. Infection had become established on all 13 inoculated trees which showed the typical black incrusted surface growth of Xylaria and the characteristic rot of the wood. In some the mycelium had spread superficially to a distance of three inches beyond the wound. The two dead checks showed no infection. It seems probable that infection had contributed to the death of the inoculated trees since 35 per cent. of the original number died during the three months as compared with a 14 per cent. death of the checks.

A scond inspection and harvest of dead trees was made at the end of the first growing season on October 14. Seven of the inoculated seedling-root trees had died in the period between July and October. All of these showed very vigorous and extensive development of black rootrot. The main axes were completely girdled and the wood was reduced in the region of the wound to the punky brittle condition characteristic of Xylaria infection. It was necessary to use care in harvesting to prevent the breaking of the roots. The mycelium had spread on the main axis in all directions from the wound and most of the branch roots were infected through their points of origin. Two other trees were practically as severely infected as the seven that died. Although the shoots were living, they could not have survived another season and would probably have died during the winter. An examination was also made at this time of the 15 remaining inoculated seedling-root trees. They were dug carefully, examined and replanted. Seven of these showed vigorous infection, three slight infection, and five no infection. The remaining check trees were free from infection and in good condition.

None of the Spy-root trees was dead at the end of the first season, but eight of them which appeared less vigorous than the others were dug, examined and

replanted. Five of these showed no infection, two showed slight infection at the wounds, and one showed moderate infection. The checks of this lot were in good condition.

A third inspection of the planting was made on September 15, 1920, after the second season's growth. Only one dead tree was found, this being one of the

FIG. 13.—Stub of a five-year-old apple tree that has died of black rootrot. The roots have been completely destroyed. Their brittleness is shown by the manner of breaking during removal. The tops were living at the beginning of the season in which death occurred.

seedling-root trees which had shown slight infection at the end of the first season. Death was clearly due to black rootrot. None of the other trees was disturbed.

The remaining trees were harvested on September 14, 1921, after the third

season of growth. Of the 14 inoculated trees on seedling-roots that remained, four had died of rootrot during the third season, two were infected but still alive, and eight were alive and free from infection. The 12 check trees were alive and healthy.

All of the original number of Spy-root trees were standing at the final harvest. Three were dead and death in all was clearly due to rootrot. Two of these had been found infected after the first season's growth and the other had not been examined then. A fourth tree showed slight infection at the wound on the branch root, but none on the main axis. It had shown slight infection after the first season's growth and there had been but little increase of the lesion in the two years that had elapsed. None of the remaining 19 inoculated trees and 8 checks showed infection and all were in good growing condition. The wounds had been completely healed with callus tissue. A summary of the data from this test is shown in Table 16.

TABLE 16

COMPARATIVE INFECTION OF APPLE TREES ON SEEDLING ROOTS AND SCION ROOTS OF NORTHERN SPY RESULTING FROM INOCULATION WITH XYLARIA MALI, CROZET, VIRGINIA, 1919 TO 1921

Type of roots	No. died during season of growth indicated			No. alive at harvest	
	First	Second	Third	Infected	Sound
Spy-roots, inoc.	0	0	3	1	19
Seedling-roots, inoc.	22	1	4	2	8
Spy-roots, check	0	0	0	0	8
Seedling-roots, check	2	0	0	0	12

	Number Inoculated	Percentage infected and:		
		Dead	Living	Total
Spy-roots	23	13.0	4.4	17.4
Seedling-roots	37	73.0	5.4	78.4

As compared with the seedling roots, the scion-roots of Northern Spy appear to have a high degree of resistance to the isolation of *X. mali* used as inoculum. Only 17.4 per cent. of the Spy roots developed infection as compared with 78.4 per cent. of the seedling roots, and 13 per cent. died from infection during three seasons as compared with 73 per cent. However, in view of the results of orchard tests to be recorded later one may well inquire into the infection and death of three of the trees on Spy roots and the escape of the others. Presumably the root systems in question should be identical in behavior since all were developed asexually from scions of the same variety. One would not expect differences in susceptibility between individual root systems of this type. It is conceivable that not all the roots purchased as Spy were of this variety but since the scion-roots of Spy have distinctive characteristics (Figure 20) this does not seem to be a strong probability. It may be true that it is more difficult to establish infection in this variety but once established infection develops normally.

Additional data bearing on the susceptibility of Spy roots were obtained at Blacksburg in a series of infection studies begun in 1922 and completed in 1927.

They have been reported in part in a previous section of this bulletin. In these tests scion roots of Spy were compared with apple seedlings grown from French and American seed. The inoculum consisted of three isolations of *X. mali* and one of *X. longeana*. The Spy-root trees were purchased from a nursery as one-year buds of Newtown Pippin, Delicious, and Winesap. The varieties were separated in the planting, but since no apparent differences existed in the behavior of the roots on them they will be treated as one in the discussion. There were 99 Spy-root trees inoculated with *X. mali*, 15 with *X. longeana*, and 15 were checks. The test was begun in April, 1922.

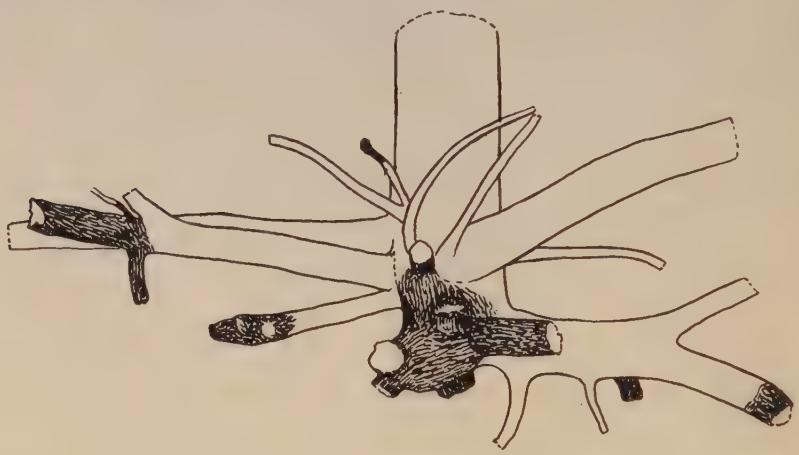

FIG. 14.—The root system of a five-year-old apple replant showing location of black rootrot infections. At least six separate infections have occurred as shown by the blackened stubs of the roots. The tops showed little evidence of disease but the tree was readily uprooted on being struck by a cultivator.

Two checks died during the first season and two others, one inoculated with *X. mali* and one with *X. longeana*, died the second season. None of these showed infection and attempts to recover the inoculum failed. No other trees died during the period of the test. They were harvested in part during the winter of 1924-1925 and in part during February, 1927. There was no infection of the lot inoculated with *X. longeana* or of the check lot. Four of those inoculated with *X. mali* showed typical symptoms of black rootrot. Five others showed some root lesions which were not typical of Xylaria infection and attempts to isolate the inoculum from them were unsuccessful. The other 89 trees had sound roots with no evidence of infection.

The behavior of the trees on seedling roots has been discussed previously and has been shown in Table 14. The American seedlings developed 36.2 per cent. infection and the French seedlings 45.4 per cent. The infection of the Spy-root trees, in striking contrast, was only 4 per cent. It seems true again that scion-roots of Northern Spy are much less susceptible to infection with the isolations of *X. mali* employed than seedling roots.

A noteworthy feature of the Spy roots was their freedom from wooly aphis. None of them showed any aphis galls. There was a considerable infestation of

the seedling roots and many were severely galled and deformed. The Spy root has been known previously for its freedom from wooly aphis infestation and it is also said to be resistant to winter injury.

Orchard Tests of Northern Spy Roots

In order to test the behavior of Spy roots under conditions of severe exposure to natural infection, plantings were made in rootrot orchards in 1919. The trees were obtained from a nursery as one-year buds on scion roots of Spy. They were set in April, 1919, as replants in two orchards where loss of trees from black rootrot had been severe, and also in an orchard slightly infected with black rootrot and rather severely infested with white rootrot. Twenty-five were set in the orchard at Staunton previously designated as Orchard A; nineteen following trees of the original stand that had recently died of black rootrot, and six following replants that had died of rootrot. The exposure to natural infection under these conditions appears to be as severe as could be obtained. The condition of the Spy-root replants was recorded in 1920 and 1921 and again in 1927. Four were dead in 1920, one from rootrot and three from mice injury. One of the latter was also affected with rootrot. Four other trees died in 1921, two of rootrot, one was killed by mice, and one died from a mechanical injury. Twelve of the remaining trees died during the interval between the inspection of 1921 and that of 1927. They were represented by blank spaces in 1927 and the cause of death could not be determined. It is believed that most of them, possibly all, died of rootrot. Only five trees of the original number were alive in 1927. These were apparently free from infection and had made good growth, although they had received but little tillage or other care during the period. In all, twenty (80 per cent.) of the Spy-root trees had died during the period from 1919 to 1927. Three of the deaths were due to rootrot (12 per cent.) and a fourth tree (16 per cent. in all) also showed infection. Five deaths (20 per cent.) were caused by mice or agencies other than rootrot, and twelve (48 per cent.) by unknown causes, rootrot being the most probable.

The behavior of the Spy-root trees in this test is not encouraging and is strongly at variance with behavior under pure culture inoculation. They showed no superiority over trees on seedling roots. Nine replants on seedling roots were set at the same time as the Spy-root trees and seven of these had died by 1927. The death of replants on seedling roots was 77.8 per cent. while that of the Spy-root replants was 80 per cent.

The planting test of Spy-root trees in an orchard at Greenwood was somewhat more encouraging than that in Orchard A, but appreciable infection and death of trees occurred. This orchard also affords a severe test. The area in which the trees were set is a hill-side that has been in orchard for about 60 years. There have been several generations of trees at many of the tree locations during this period. The original planting was of Fall Cheese, Mother, Limbertwig, Pilot, and Northern Spy. As these trees died they were replaced with Newtown (Albemarle) Pippin. Many of the trees of the second planting have died and have in turn been followed by one or more replants. The Spy-root replants were set in places where it had been impossible to grow trees because of rootrot. According to the owner, every replant set prior to the test had died at ages between three and seven years. Nineteen Spy-root replants were set in 1919 and 20 more were set in 1920. Five trees of the first lot had died by the fall of 1927. Two of these died of rootrot in 1924. Two died in either 1925 or 1926, and one died of rootrot in 1927. The writer has not seen all trees removed but the owner states that all

of them died of rootrot. Three of the remaining trees of this lot appeard to be weak in 1927, but the other 11 appeared healthy and vigorous.

Of the 20 Spy-root replants set in 1920, six had died by 1927. One died in 1924, four in either 1925 or 1926, and one in 1927. These deaths were likewise due to rootrot. The remaining fourteen trees of this lot all appeared healthy and vigorous.

The deaths in the planting of 1910 have been 26.3 per cent. and those in the planting of 1920 have been 30 per cent

The orchard owner considers the Spy roots to be distinctly superior to seedling roots. He would have expected to lose 100 per cent. of trees on seedling roots in the locations where the Spy roots were set and does not consider the loss of one-third of them in eight or nine years to be excessive under the conditions.

FIG. 15.—A, Rot of woody parts of the roots of two apple seedlings five months after inoculation with *X. mali*. There was little superficial evidence of infection. B, A localized lesion on an apple root resulting from natural infection by *X. mali*.

The third planting consisted of fourteen Spy-root trees set in 1919 in an orchard near Afton. Tree loss here has been due chiefly to white rootrot but black rootrot was also present in some degree. All trees were living at the end of the season of 1927 and were in good health and vigor. The owner of the orchard is well pleased with the behavior of the Spy-root trees and now uses them exclusively for replanting.

Infection Studies With Seedlings of Known Maternal Parentage

Tests of apple seedlings of known maternal parentage have been conducted on a considerable scale in the hope that a constant and appreciable degree of resistance might be found to exist in some varieties. The seed were from open

pollinated fruits. A majority of the seedlings were grown at Arlington Farm by Guy E. Yerkes of the U. S. Department of Agriculture, who kindly supplied them for our work. Others were grown by us from seed which we had harvested.

Seedlings of ten varieties grown in 1924 were used for inoculation tests in 1925. Pure culture inoculum of an isolation of *Xylaria mali* was inserted into wounds after the manner of previous work on March 24 and planting was completed on the same day. A drought existing at planting time which continued for several weeks was evidently unfavorable for infection. Only one of more than 100 seedlings became infected. This was one of ten seedlings of the Lowry variety. The tree grew well in the early season and developed into full leaf with terminal shoots ten inches long. The top withered and died in June and the roots, when examined on July 2, were found to be girdled by the mycelium of the pathogen. Death was clearly due to infection from the inoculum.

The best of the remaining seedlings were used for a second test in 1926. An isolation of *X. mali* was inserted into wounds in the usual manner on April 5 and the trees were planted at Blacksburg on the same day. No checks were employed because of the limited number of individuals. Drought conditions again prevailed during the early season and the growth of tops was rather poor in consequence. All the trees survived until fall when they were removed and examined for infection. Such infections as occurred were limited in extent. There was little development of the surface crust but typical rot of the wood adjacent to the wound had occurred in some individuals and mycelium of *X. mali* developed on such wood in moist chamber. The results of the test are shown in Table 17. All lots developed some infection, but the nursery seedlings and the varieties Northern Spy, Limbertwig, and Ben Davis developed appreciably less than did Grimes, Lowry, and Winesap.

TABLE 17
RESPONSE OF SEEDLING APPLE TREES OF KNOWN MATERNAL PARENTAGE TO INOCULATION WITH X. MALI IN 1926

Variety	Number of trees		Percentage infected
	Inoculated	Infected	
Ben Davis	16	4	25.0
Grimes	7	3	42.9
Limbertwig	11	2	18.2
Lowry	8	4	50.0
Northern Spy	32	4	12.5
Nursery seedlings*	15	1	6.7
Winesap	8	5	62.5

*Commercial seedlings of unknown parentage

An additional test in 1926 included one-year-old seedlings of 34 varieties and of an additional species, *Malus zumi* Rehd. They were inoculated as in the preceding test and were planted in part at Blacksburg on May 13 and in part at Staunton on May 4. The lateness of the planting dates together with the dry weather resulted in the death of considerable numbers of seedlings. The unfavorable growth conditions may have aided infection but it seems equally probable that higher percentages of infection might have resulted with more moisture at planting time. The data should be criticized with these facts in mind. They are shown in Table 18.

The results obtained at the two stations are as a whole in rather fair agreement, although certain varieties show rather wide discrepancies. If the percentages of infection for all lots are averaged that at Blacksburg is 16.4 and that at Staunton 17.2. The combined data in Table 19 presents a fair average picture of the behavior of the varieties under the conditions of the tests. It seems to be true that seedlings of Winter Banana, Ben Davis, Mammoth Grimes, King David, and Delicious are among the most resistant tested and that those of Yellow Newtown and Northern Spy are most susceptible. *Malus zumi* also appears to be quite susceptible.

TABLE 18

RESPONSE OF SEEDLINGS OF APPLE TREES OF KNOWN MATERNAL PARENTAGE TO INOCULATION WITH X. MALI IN 1926. THE SYMBOL (Y) INDICATES SEEDLINGS GROWN BY MR. YERKES AND (H) INDICATES THOSE GROWN BY MR. HURT

Variety or species	Staunton				Blacksburg			
	Set	Died	Inf.	% Inf.	Set	Died	Inf.	% Inf.
Ben Davis	29	1	0	none	16	4	1	6.3
Ben Hur	32	6	2	6.3				
Black Ben Davis	53	12	7	13.2				
Delicious (Y)	102	18	6	5.9	24	2	3	12.5
Delicious (H)	30	1	1	3.3				
Fameuse	29	5	4	13.8	29	12	6	20.7
Florence	23	15	5	21.7				
Gano	11	4	3	27.3				
Grimes	22	9	4	18.2	15	1	3	20.0
Jonathan	81	46	20	24.7	21	2	2	9.5
Julien le Paulmier	95	29	17	17.9	48	14	17	35.4
King David	27	7	1	3.7				
Kinnard	27	9	4	14.8				
Lawver	27	5	4	14.8	30	6	5	16.7
Lowry	63	25	9	14.3				
McIntosh	32	29	13	40.6	31	9	5	16.1
Magnate	56	26	10	17.9				
Malus zumi	28	14	13	46.4	30	12	6	20.0
Mammoth Grimes	30	9	1	3.3				
Missouri Pippin	90	40	6	6.7	30	12	9	30.0
Northern Spy (Y)	18	5	3	16.7				
Northern Spy (H)	13	13	7	53.8				
Passe Reine	99	28	10	10.1	20	4	1	5.0
Pilot	26	6	3	11.5				
Red Astrachan	47	10	7	14.9	18	1	1	5.6
Red Siberian	29	19	8	27.6	15	1	2	13.3
Rome	22	11	3	13.6	17	3	2	11.8
Rouge du Landel	42	11	3	7.1	32	10	4	12.5
Senator	25	17	5	20.0				
Spitzenburg	33	10	4	12.1	31	6	8	25.8
Tolman	105	13	8	7.6	30	6	8	26.7
Wealthy	91	37	13	14.3	42	17	9	21.4
Winesap	82	14	7	8.5				
Winter Banana	55	3	1	1.8				
Yellow Newtown	14	6	5	35.7				
Yellow Siberian	25	12	8	32.0	31	5	6	19.4
York Imperial	19	9	4	21.1				

Certain individuals that had developed no infection in these trials and which were of good vigor and type were selected for further tests in the greenhouse. Eight trees each of 17 varieties were selected and an additional variety, Delicious, was represented by six trees. The trees were inoculated through wounds and were planted in a deep bed of soil in the greenhouse on December 20, 1926. Two isolations of *X. mali* were employed, one from Staunton and one from Greenwood. The trees were kept well watered throughout the test and such deaths as occurred were clearly due to the pathogen. Dead trees were removed at intervals during the course of the test which was completed on May 13, 1927, when the remaining trees were dug and examined.

TABLE 19

PERCENTAGES OF INFECTION WITH X. MALI ON APPLE SEEDLINGS IN 1926. COMBINED DATA FOR BOTH STATIONS

Variety	% Inf.	Variety	% Inf.
Winter Banana	1.8	Wealthy	16.5
Ben Davis	2.2	Fameuse	17.2
Mammoth Grimes	3.3	Magnate	17.9
King David	3.7	Spitzenburg	18.8
Ben Hur	6.3	Grimes	18.9
Delicious	6.4	Senator	20.0
Winesap	8.5	York Imperial	21.1
Passe Reine	9.2	Jonathan	21.6
Rouge du Landel	9.5	Florence	21.7
Pilot	11.5	Red Siberian	22.7
Tolman	11.8	Julien le Paulmier	23.8
Red Astrachan	12.3	Yellow Siberian	25.0
Missouri Pippin	12.5	Gano	27.0
Rome	12.8	McIntosh	28.6
Black Ben Davis	13.2	Northern Spy	32.3
Lowry	14.3	*Malus zumi*	32.8
Kinnard	14.8	Yellow Newtown	35.7
Lawver	15.8		

Of the two cultures employed, that from Staunton was decidedly the more virulent. Four trees of each variety, except Delicious where three were used, were inoculated with this culture, a total of 71 trees. Ten of these died of rootrot during the five-month period of the test, 20 developed severe infection, 9 slight infection, and 32 were free from infection. Six of the ten deaths occurred within two months of the date of inoculation, during the seventh and eighth weeks, and the other four occurred within three months of inoculation. Approximately one-half of the trees showing severe infection had developed the typical black crust on the surface of the roots. The others showed little superficial evidence of infection but there was appreciable invasion and rotting of the woody parts of the roots (Figure 15, A). It is believed that all trees recorded as severely infected would have succumbed to rootrot in the course of time. It seems evident that the survival and freedom from infection shown by such trees in the test of the preceding year was due not to resistance but to disease escape occasioned by conditions unfavorable for infection and development of the pathogen.

The behavior of varieties in the lot inoculated with the isolation from Staunton is shown in Table 20. One or more trees of each variety developed infection except those of the varieties Passe Reine and Rouge du Landel, and conversely one or more trees of each variety escaped infection except those of the varieties McIntosh, Rome, and Wealthy. All the trees of the latter variety developed severe infection and one died. Two trees of the varieties Lawver and Rome died and one additional of each developed severe infection. While the test is too limited in scope to warrant conclusions as to the resistance or susceptibility of the varieties in general it may have significance in connection with other tests. There are some points of agreement in the grouping of varieties in this test and in that conducted in the open in 1926 (Table 19). It may be of significance that the varieties Ben Davis, Missouri Pippin, Passe Reine, Rouge du Landel, and Tolman appear among the seven least susceptible varieties of those represented in both lists.

The isolation from Greenwood proved much less virulent than that from Staunton. Only one tree of the 71 inoculated died of rootrot. This was one of four trees of Ben Davis. It died three months after inoculation. When harvested six additional trees showed severe infection, four slight infection, and 60 no in-

TABLE 20

RESPONSE OF APPLE SEEDLINGS OF KNOWN MATERNAL PARENTAGE TO INOCULATION WITH XYLARIA MALI, STAUNTON ISOLATION, IN THE GREENHOUSE

Variety	Number of trees				Per cent. severe infection
	Dead and infected	Severe infection	Slight infection	No infection	
Passe Reine	0	0	0	4	0
Rouge du Landel	0	0	0	4	0
Missouri Pippin	0	0	1	3	0
Yellow Siberian	0	0	2	2	0
Tolman	0	1	0	3	25
Ben Davis	0	1	1	2	25
Red Siberian	0	1	1	2	25
Jonathan	1	0	0	3	25
Grimes	1	0	1	2	25
Julien le Paulmier	1	1	0	2	50
Winesap	1	1	1	1	50
Delicious	0	2	0	1	67
Red Astrachan	0	3	0	1	75
McIntosh	0	3	1	0	75
Fameuse	1	2	0	1	75
Lawver	2	1	0	1	75
Rome	2	1	1	0	75
Wealthy	1	3	0	0	100

fection. The response of varieties was as follows: two trees of Red Siberian and one each of Ben Davis, Jonathan, McIntosh, Rouge du Landel, and Winesap developed severe infection; two of Passe Reine, and one each of Ben Davis and Missouri Pippin developed slight infection.

A further test of certain individuals that had survived this greenhouse test was conducted in the open in 1927. From one to three trees of each of 13 varieties, 26 trees in all, which had developed no infection were selected and were inoculated with chips of wood from root lesions on trees that had been inoculated with the Staunton isolation. The roots were pruned to provide cuttings for propagation and the inoculum was inserted in a wound and then wrapped with moist cotton. The tops were pruned vigorously because of the lateness of the season and the loss of roots from pruning. The trees were inoculated and planted on May 14, and all but one survived transplanting and were alive at the end of the season. Thirteen of the 26 individuals developed severe infection, three moderate infection, one slight infection, and nine no infection. In no instance did all trees of any variety show complete freedom from infection.

The response of varieties was as follows: two trees of the varieties Julien le Paulmier and Yellow Siberian, and one of Grimes, McIntosh, Red Astrachan, Red Siberian, and Wealthy showed no infection; one of Red Siberian showed slight infection; one of Fameuse, Grimes, and Yellow Siberian showed moderate infection; two of Delicious, Passe Reine, and Rouge du Landel, and one of Julien le Paulmier, Fameuse, Jonathan, McIntosh, Red Siberian, Rome, and Wealthy showed severe infection. When one considers that all of these trees had survived two previous inoculation trials without developing infection and that 50 per cent. developed severe infection in the third trial, it seems apparent that faulty technique or conditions of environment and growth were most likely responsible for failure in the earlier tests. A doubt that the survivors of the third test will continue to maintain their apparent resistance in future tests may also be expressed. A fourh test is now under way and additional plants of the non-infected survivors, propagated from root cuttings, are also under test.

It is to be noted that the third test differed from the former two in that chips of affected wood were used as inoculum instead of mycelium from culture grown in media. It has been apparent in various tests that failure to infect with mycelium may result from rapid drying of the inoculum when soil moisture is deficient. There is less likelihood of such failure if chips of wood are employed, but the

FIG. 16.—Invasion of root and crown of Norway maple resulting from inoculation with *X. mali.* The inoculum was inserted in the root which is completely destroyed. The margin of the affected area is clearly defined. Inoculated April, 1922, photographed February, 1925.

pathogen in such chips is obviously not in pure culture. Various fungi may follow *X. mali* in affected wood. *Trichoderma koningi* is an especially common contaminant. This species, as well as others that have been isolated from wood invaded by *X. mali,* is saprophytic and its presence presumably does not modify the course of disease. It is conceivable that the exposure when wood chips are inserted into wounds in the root is more severe than is normally encountered in nature but the search for a resistant stock should employ stringent methods.

DISCUSSION

Although the general fact of the parasitism of *X. mali* for apple is fully established, and no doubt exists as to the causative agent in the black rootrot disease, little is known as yet of the more intimate details of host-parasite relations. Infection with typical symptoms of the disease has devloped in a considerable number of individuals from inoculation with the fungus in pure culture and the inocu-

FIG. 17.—The black incrusted appearance of roots affected with black rootrot is well shown in this scion-rooted tree of Yellow Newtown which was inoculated with *X. mali* on the uppermost right-hand root.

lum has been recovered in numerous instances. Variable results have, however, frequently been obtained. Attempts to produce infection have sometimes failed or have been only partially successful, and virulence in experimental trials has usually been lower than that which appears to exist under conditions of natural exposure to infection. The experience with certain seedlings that survived two

infection trials and succumbed in the third is an instance in point as is also the contrasting behavior of scion-roots of Northern Spy in experimental tests and orchard trials. Inconsistencies of this sort may be explained by assuming the technique of inoculation to be at fault or they may be determined by factors of environment or by the existence of strains of the fungus of distinct pathogenicity. These and other possibilities should receive consideration in further studies.

FIG. 18.—Appearance of diseased and normal replants of the same age. The tree at the left which was practically dead of black rootrot was readily uprooted.

Little is known of the factors that favor or hinder infection and development of black rootrot. It appears to exist in all types of orchard soils with little re-

gard to general qualities. This is indicated by its geographic occurrence in Virginia and other states. High organic content appears to be favorable for disease as does also high soil moisture which usually accompanies the former. It occurs, however, without regard to topography; it may be fully as prevalent on the knolls as in the draws. Such ranges of soil acidity as occur in the orchard soils of Virginia seem to have little influence on the disease. The pH of both top-soil and sub-soil was determined for the orchards included in the survey of 1926 and appreciable rootrot losses were found in soils ranging from neutrality to strong acidity. No distinctly alkaline soils were found. It seems to be true that the presence or absence of rootrot in the orchard is determined not by the qualities of the soil but by the presence or absence of the pathogen.

The existence of strains of *X. mali* that are distinct in pathogenicity is indicated by the behavior of the Staunton and Greenwood isolations in the greenhouse tests of 1926-27 (Table 20) and by other observations. Strains distinctive in appearance on culture media have been isolated from various localities. Such strains may conceivably have distinct potentialities as parasites, and their existence must receive consideration in the study of measures of control.

The tangible results of the studies of susceptibility of types of roots have been negative rather than positive, but they appear to indicate the trend that should be pursued further in this phase of the problem. It seems improbable that open-pollinated seed of any of the standard varieties will furnish stocks of desired uniformity and resistance to rootrot. Self-pollinated seed, even though they should prove superior in both respects, could not be supplied in quantity to meet trade requirements under existing conditions of orchard management. It would appear more profitable to focus attention on the behavior of individuals without regard to parentage, in the expectation that asexual methods of propagation could be employed for any that exhibited the desired qualities. More extended study of outstanding seedling individuals may readily discover one or more that meets the requirements. The root systems of particularly long-lived trees, and especially those that have survived in orchards where rootrot is prevalent, should offer a further likely source of material for study. Healthy trees of the Northern Spy variety, approximately 60 years old, are standing in an orchard at Greenwood where rootrot losses have been severe. The more superficial roots of certain trees were examined and were found to have the characteristics of scion roots of that variety. The longevity of these trees may be due to the resistance of their scion roots.

Inquiry into the response of scion roots of varieites other than the limited number that we have tested may also prove of value. Certain varieties produce roots readily from scion wood, especially when grafted on a short nurse root. Other varieties may be induced to do so by special methods but their root systems are apt to be scanty and inadequate for the support of a tree of normal vigor and size. Northern Spy is one of the outstanding varieties from this standpoint. Its scions root readily when grafted to nurse roots and planted deeper than is usually done, or when layered in trenches. The varieties, Baldwin, Black Ben Davis, Delicious, Duchess, McIntosh, Maiden Blush, Mother, and Wilson Red June, as well as certain others, also produce good scion roots. Although trees produced by such methods are more expensive their cost need not be at all prohibitive.

In addition to the partial resistance to rootrot which it undoubtedly possesses, the scion root of Northern Spy has other desirable qualities. In our tests at Blacksburg, where it was grown in comparison with seedlings from imported and domestic seed, it exhibited marked resistance to wooly aphis injury. Many of the

seedlings were severely infested and deformed while none of the Spy roots showed injury. It has also appeared to have greater resistance to drought than the average of seedling roots. Although its use for replanting in rootrot orchards or for original planting on land where rootrot may be expected to occur cannot be advised without qualification, it may be expected to prove somewhat superior

FIG. 19.—Infection of a scion rooted tree of Red Astrachan resulting from inoculation with *X. mali*. The inoculum was inserted in the root at the right which broke at that point during removal. The margins of the affected areas are indicated by white lines. Inoculated May, 1917; harvested and photographed April, 1919.

to the seedling stocks commonly employed under average conditions. A stock of greater resistance is needed, however, for a satisfactory solution of the problem and the search for such a stock should continue. It may conceivably be found in the roots of a seedling or in scion roots of a variety.

Although we have considered the development of a resistant root stock to offer the most likely and satisfactory solution of the problem of control there are other possibilities which should not be overlooked. Our studies of these have not been encouraging but have been quite limited. Some of the more common disinfectants applied to the soil before replanting proved of no benefit when tested on a very limited scale. Modification of the soil reaction through applications of

FIG. 20.—Scion roots of Northern Spy have a distinctive and characteristic appearance. Burr-knots are formed and the rootlets commonly occur in groups.

lime has given no consistent benefit. Attempts to extend the life of affected trees by planting young trees around them and inarching into the trunk and scaffold limbs have been unavailing. Such benefits as may be obtained from practices designed to restrict the spread of infection, such as avoidance of plowing, destruction of mice, and prompt removal of diseased trees, are not apt to be of sufficient magnitude to satisfy the requirements. They may delay in some measure the spread of infection to trees of the initial stand but they will not solve the problem of replanting. A resistant rootstock or a thorough sterilization of the soil would seem to offer the best possibilities in this respect.

SUMMARY

The fungus which causes black rootrot of apple is described as *Xylaria mali*. The species appears to be identical with that which Ellis erroneously referred to *X. digitata*, a name which is properly applied to the European species. Distinguishing features are the richly branched stromata, the compressed, blunt tips of the stromatic arms; the pigmented area in which the perithecia are deeply embedded, and the ascospores which measure 5-6 x 12-16μ.

Numerous collections of the species have been made on apple in Virginia and its occurrence in the states of West Virginia, North Carolina, South Carolina, Kentucky, Tennessee, Indiana, Illinois, and Arkansas is attested.

Xylaria polymorpha occurs rarely on apple in Virginia but from reports is more common in northern states, especially New York. It is not so vigorously parasitic as *X. mali*.

X. mali grows readily in culture on a variety of media and produces stromata which bear conidia. Attempts to induce formation of perithecia and ascospores in culture have been fruitless.

Diagnostic symptoms of black rootrot are, black mycelial incrustations on the surface of infected roots, the brittle, punky character of the invaded root, and the fruiting of the pathogen on the trunk at the ground line during late stages of disease.

Orchard studies in the Shenandoah Valley indicate black rootrot to be the most important single cause of apple tree death. In the average infested orchard the loss has equalled 18 per cent. at 21 years of age. Detailed studies in three orchards have shown tree losses of 28 per cent. at 25 years, of 21 per cent. at 21 years, and of 24 per cent. at 15 years. Few trees set as replants following trees dead of rootrot survive until bearing age.

Studies of incidence and duration of disease, of the fruiting of the pathogen, and of the history of replanting are reported in detail.

Under comparable conditions losses of trees of the York Imperial variety greatly excedeed those of Ben Davis.

Infection and typical symptoms of disease have been produced in apple through contact with infected root pieces, by planting in infested soil, and in numerous instances through inoculation with *X. mali* in pure culture.

Vigorous development of infection has occurred in Norway maple and Mahaleb cherry. Less vigorous infection resulted in elm, honey locust, *Pyrus communis* and *P. ussuriensis*. No infection developed in ash, butternut, black walnut, chestnut, black locust, silver maple, or box elder.

Scion roots of the Northern Spy apple have shown considerable resistance to infection in inoculation tests but when set as replants under severe exposure to infection appreciable percentages have died of rootrot.

Studies of the susceptibility of commercial seedlings and those of a considerable number of varieties of known maternal parentage indicate such resistance as may exist to be a property of individuals rather than of varieties.

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